Phosphodiesterases as therapeutic targets for respiratory diseases

Haoxiao Zuo [a,c,*], Isabella Cattani-Cavalieri [a,b,d], Nshunge Musheshe [a], Viacheslav O. Nikolaev [c,e], Martina Schmidt [a,b]

[a] *Department of Molecular Pharmacology, University of Groningen, the Netherlands*
[b] *Groningen Research Institute for Asthma and COPD, GRIAC, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands*
[c] *Institute of Experimental Cardiovascular Research, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany*
[d] *Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[e] *German Center for Cardiovascular Research (DZHK), 20246 Hamburg, Germany*

ARTICLE INFO

ABSTRACT

Chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, affect millions of people all over the world. Cyclic adenosine monophosphate (cAMP) which is one of the most important second messengers, plays a vital role in relaxing airway smooth muscles and suppressing inflammation. Given its vast role in regulating intracellular responses, cAMP provides an attractive pharmaceutical target in the treatment of chronic respiratory diseases. Phosphodiesterases (PDEs) are enzymes that hydrolyze cyclic nucleotides and help control cyclic nucleotide signals in a compartmentalized manner. Currently, the selective PDE4 inhibitor, roflumilast, is used as an add-on treatment for patients with severe COPD associated with bronchitis and a history of frequent exacerbations. In addition, other novel PDE inhibitors are in different phases of clinical trials. The current review provides an overview of the regulation of various PDEs and the potential application of selective PDE inhibitors in the treatment of COPD and asthma. The possibility to combine various PDE inhibitors as a way to increase their therapeutic effectiveness is also emphasized.

Contents

1. Introduction

Respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma are among the leading causes of morbidity and mortality today. COPD and asthma combined affect at least 300 million people worldwide, making investigation of more therapeutic targets and the development of effective drugs a relevant task in the treatment of these respiratory diseases (Vogelmeier et al., 2017).

COPD and asthma are characterized by airway obstruction, chronic inflammation, and airway remodeling. Despite both COPD and asthma being characterized by airway obstruction, the airway obstruction in COPD is progressive and not fully reversible, while that in asthma is reversible by bronchodilators and is associated with airway hyperresponsiveness (Guerra, 2009; Hogg & Timens, 2009; Meurs, Gosens, & Zaagsma, 2008). In addition, airway inflammation in COPD is characterized by an increased number of neutrophils, macrophages and CD8+ T-lymphocytes, while that in asthma is characterized by the infiltration of eosinophils, mast cells and CD4+ T-lymphocytes (Mauad & Dolhnikoff, 2008; Vogelmeier et al., 2017; Welte & Groneberg, 2006).

Currently, therapeutic management of COPD relies mainly on the use of bronchodilators (β_2-adrenoceptor (β_2-AR) agonists, anticholinergics and theophylline), and a combination therapy of inhaled corticosteroid plus long-acting β_2-AR agonists. In patients with severe COPD associated with bronchitis and a history of frequent exacerbations, the phosphodiesterase (PDE) 4 inhibitor roflumilast is typically used as an add-on treatment to the above mentioned therapies (Giembycz & Maurice, 2014). In asthma treatment and/or management, the combination therapies of inhaled corticosteroid and short-acting β_2-AR agonists or long-acting β_2-AR agonists are used to control symptoms and relieve bronchoconstriction (Dekkers, Racké, & Schmidt, 2013; Reddel et al., 2015; Silva & Jacinto, 2016). In addition to current therapies in asthma treatment, oral roflumilast has been proposed as a beneficial add-on therapy for use in patients with moderate-to-severe asthma (Beghè, Rabe, & Fabbri, 2013).

In this review, we discuss several PDE subtypes and how their selective inhibitors are of interest for therapeutic application in COPD and asthma treatment. The possibility to combine various PDE inhibitors to increase their therapeutic effectiveness is also emphasized.

2. Systematic overview of the PDE superfamily

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are ubiquitous second messengers. cAMP and cGMP play important roles in regulating numerous cellular functions in physiology and pathology of the lung, including but not limited to the airway smooth muscle (ASM) tone, cell proliferation, differentiation, apoptosis, migration, secretion of inflammatory mediators, deposition of extracellular matrix, and the maintenance of the endothelial and epithelial barrier (Beavo & Brunton, 2002; Billington, Ojo, Penn, & Ito, 2013; Sayner, 2011; Zhang, Zhang, Qi, & Xu, 2016).

Following activation of adenylyl cyclases (ACs) or guanylyl cyclases, cAMP and cGMP are synthesized from adenosine triphosphate and guanosine triphosphate, respectively (Omori & Kotera, 2007). Subsequently, cAMP and cGMP bind to specific intracellular effector proteins, such as: cyclic nucleotide-gated ion channels, cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), exchange proteins directly activated by cAMP (Epacs) (Oldenburger, Maarsingh, & Schmidt, 2012; Omori & Kotera, 2007; Pfeifer, Kilić, & Hoffmann, 2013) and the most recently described Popeye domain containing proteins which bind cAMP with a high affinity (Schindler & Brand, 2016). The intracellular cyclic nucleotide concentrations are substantially determined by PDEs (shown in Fig. 1), which hydrolyze cAMP and cGMP and prevent it from diffusing to other compartments thereby compartmentalizing the cyclic nucleotide signal.

The superfamily of PDEs is composed of 11 families with a distinct substrate specificity, molecular structure and subcellular localization (Omori & Kotera, 2007). In this article, some of the key features of the PDE superfamily are discussed, with the reader being referred to more specific reviews for future insights in the molecular mechanisms of the regulation of PDE sutypes (Abbott-Banner & Page, 2014; Omori & Kotera, 2007; Page, 2014; Page & Spina, 2012). Each PDE family has at
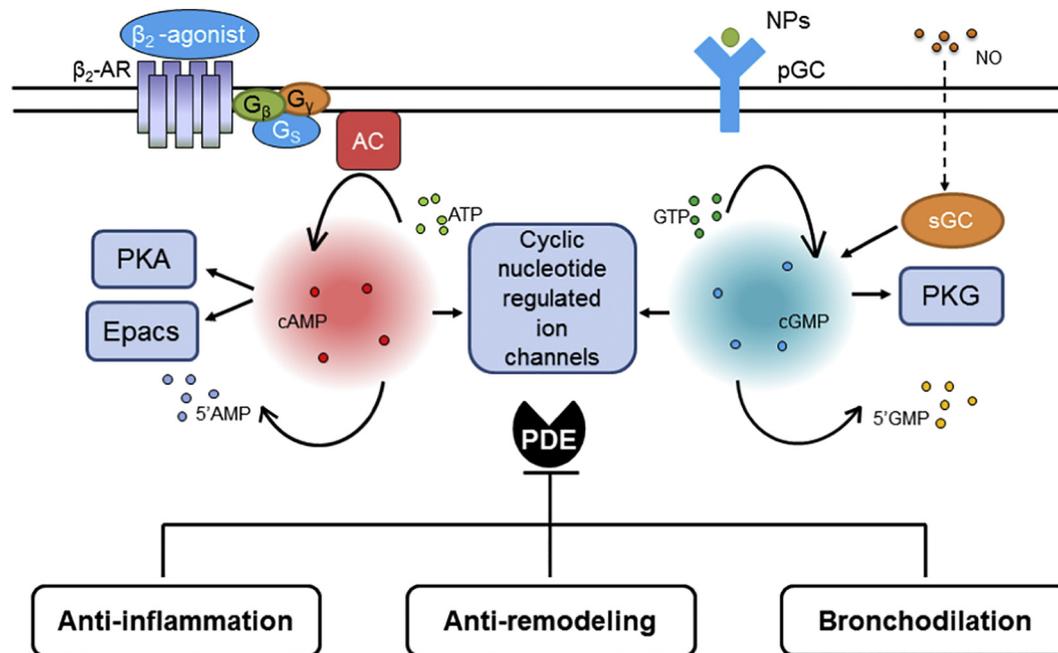
Fig. 1. Cyclic nucleotides signaling in the lung. cAMP is synthesized by adenylyl cyclase (AC) from adenosine triphosphate. AC is activated by a range of molecules via stimulatory heterotrimeric G-protein subunits. Similarly, cGMP is synthesized by guanylate cyclase (GC) from guanosine triphosphate. Soluble GC is directly activated by nitric oxide, whereas particulate GC is activated by natriuretic peptides. Cyclic nucleotides binding proteins are cyclic nucleotide-gated ion channels, cAMP-dependent protein kinase A, cGMP-dependent protein kinase G, and exchange proteins directly activated by cAMP (Epacs). cAMP and cGMP are hydrolyzed by phosphodiesterases. In the lung, PDE inhibition exerts anti-inflammatory, anti-remodeling and bronchodilator effects. β_2-AR, β_2-adrenoceptor; AC, adenylyl cyclase; ATP, adenosine triphosphate; GC, guanylate cyclase; GTP, guanosine triphosphate; sGC, soluble guanylate cyclase; NO, nitric oxide; pGC, particulate guanylate cyclase; NPs, natriuretic peptides; PKA, cAMP-dependent protein kinase A; Epacs, exchange proteins directly activated by cAMP; PKG, cGMP-dependent protein kinase G; PDE, phosphodiesterases.

least one (e.g. *Pde5a*) and often multiple coding genes, resulting in the mammalian PDE superfamily being composed of more than 21 genes. (Omori & Kotera, 2007; Page & Spina, 2012). Moreover, most PDE encoding genes have distinct promoters, and multiple transcriptional products which are generated by alternative splicing, resulting in nearly 100 different PDE messenger RNAs (Conti & Beavo, 2007; Otero et al., 2014).

Based on the substrate preferences for either cAMP or cGMP, PDEs are sub-divided into 3 groups: the cAMP-specific PDEs (PDE4, PDE7, and PDE8), the cGMP-specific PDEs (PDE5, PDE6, and PDE9) and dual-specific PDEs which hydrolyze both cAMP and cGMP (PDE1, PDE2, PDE3, PDE10 and PDE11). It is worth noting that some dual-specific PDEs play vital roles in the crosstalk between cAMP and cGMP. For instance, PDE2 is referred to as a cGMP-stimulated cAMP PDE. When cGMP binds to the amino terminus of the allosteric regulatory site known as the GAF-B domain of PDE2, the hydrolysis rate of cAMP is increased by 10-fold, and therefore cGMP is able to negatively regulate the cellular concentration of cAMP via PDE2 (Martinez et al., 2002; Pavlaki & Nikolaev, 2018). Another PDE involved in the cAMP and cGMP crosstalk is PDE3, which is termed a cGMP-inhibited cAMP PDE. Due to a higher affinity and lower catalytic hydrolysis rate for cGMP compared to cAMP, cGMP acts as a competitive inhibitor of cAMP hydrolysis by PDE3 (Degerman, Belfrage, & Manganiello, 1997; Shakur et al., 2001). In Table 1, the PDE substrate specificities, their expression profile in the lung, and prominent PDE inhibitors are summarized.

3. PDE3

PDE3 is transcribed from two genes, PDE3A and PDE3B, which show high affinity to both cAMP and cGMP. Due to a lower V_{max} value for cGMP compared to that for cAMP, cGMP functions as a competitive inhibitor for cAMP hydrolysis by PDE3 and therefore PDE3 is referred to as a cGMP-inhibited cAMP PDE (Omori & Kotera, 2007). Three iso-forms are encoded by *Pde3a*, PDE3A1 to PDE3A3, and only one isoform is described for PDE3B (Movsesian, Ahmad, & Hirsch, 2018). PDE3A is abundant in the cardiovascular system, including the myocardium, arterial and venous smooth muscle, bronchial, genitourinary and gastrointestinal smooth muscle as well as the epithelium, megakaryocytes, and oocytes, while PDE3B is highly expressed in adipose tissue (Reinhardt et al., 1995). In the lung, PDE3 was detected in alveolar macrophages, lymphocytes, monocytes, platelets, endothelial cells, as well as in epithelial cells and ASM cells (Beute et al., 2018; Chung, 2006; Gantner, Schudt, Wendel, & Hatzelmann, 1999; Wright, Seybold, Robichaud, Adcock, & Barnes, 1998; Zuo et al., 2018). A substantial body of evidence suggests that PDE3 inhibitors including siguazodan, SK&F94120 and org9935 are potent relaxants in ASM (Bernareggi, Belvisi, Patel, Barnes, & Giembycz, 1999; Nicholson et al., 1995; Torphy et al., 1993). Despite detecting PDE3 in T-lymphocytes, however, PDE3 inhibition has been found to have little effect on T-cell proliferation and cytokine generation (Giembycz, Corrigan, Seybold, Newton, & Barnes, 1996).

Recently, Beute and co-workers investigated the role of PDE3 in an acute house dust mite-driven (HDM-driven) allergic airway inflammation mouse model. Using a targeted deletion of *Pde3a* or *Pde3b* gene in mice, the number of inflammatory cells and the concentration of pro-inflammatory cytokine were evaluated. They showed that the number of eosinophils in bronchoalveolar lavage (BAL) fluid was significantly decreased in both HDM-treated PDE3A-/- mice and PDE3B-/- mice when compared to HDM-treated wild type (WT) mice. Other inflammatory cells, including T-lymphocytes, neutrophils, macrophages followed roughly the same pattern. Moreover, the proportion of IL-5- and IL-13-positive CD4+ T cells in BAL fluid was significantly decreased in HDM-

Table 1
The PDE superfamily: Substrate preference, lung expression profile and most commonly used inhibitors.

PDE family	Subfamilies	Substrate	Lung cell types	PDE inhibitor	References
PDE1	PDE1A PDE1B PDE1C	cAMP/cGMP	Pulmonary arterial smooth muscle cells; epithelial cells; fibroblasts; macrophages;	8-methoxymethyl-IBMX; vinpocetine; nimodipine; IC86340; IC295;	Brown et al. (2007), Dunkern et al. (2007), Kogiso et al. (2017), Murray et al. (2007), Schermuly et al. (2007)
PDE2	PDE2A	cAMP/cGMP	Pulmonary arterial smooth muscle cells; endothelial cells; macrophages	BAY 60-7550; PDP; EHNA; IC933; oxindole; ND-7001;	(Bubb et al. (2014), PDE2 inhibition, 2013, Snyder, Esselstyn, Loughney, Wolda, and Florio (2005), Witzenrath et al. (2009)
PDE3	PDE3A PDE3B	cAMP/cGMP	Bronchial epithelial cells; airway smooth muscle cells; vascular smooth muscle cells; fibroblasts; T-lymphocytes; macrophages	Olprinone; cilostamide; milronone; cilostazol; milrinone; siguazodan; enoximone; motapizone; SK&F94120; org9935;	Giembycz et al. (1996), Hwang et al. (2012), Mokra, Drgova, Pullmann, and Calkovska (2012), Selige et al. (2010), Zuo et al. (2018))
PDE4	PDE4A PDE4B PDE4C PDE4D	cAMP	Inflammatory cells, fibroblasts, pulmonary arterial smooth muscle cells; airway smooth muscle cells; epithelial cells; endothelial cells	Rolipram; roflumilast; cilomast; RP73401; Ro20-1724; CHF6001; GPD-1116; ASP3258; YM976;	Armani et al. (2014), Barber et al. (2004), Belleguic et al. (2000), Hatzelmann and Schudt (2001), Kubo et al. (2011), Millen et al. (2006), Mori et al. (2008), Sachs et al. (2007)
PDE5	PDE5A	cGMP	Airway smooth muscle cells; vascular smooth muscle cells; epithelial cells; fibroblasts	Zaprinast; DMPPO; sildenafil; tadalafil; vardenafil; dipyridamole; E4021; avanafil;	Aldashev et al. (2005), Dent et al. (1998), Sebkhi et al. (2003), Selige et al. (2010)
PDE6	PDE6A PDE6B PDE6C PDE6D	cGMP	Epithelial cells; other cell types largely unknown	Zaprinast; DMPPO; sildenafil; vardenafil;	Nikolova et al. (2010), Zhang et al. (2005)
PDE7	PDE7A PDE7B	cAMP	Inflammatory cells; bronchial epithelial cells; airway smooth muscle cells; lung fibroblasts; pulmonary arterial smooth muscle cells; vascular endothelial cells	BRL 50481; IC242; T-2585; compound 21a;	Gantner et al. (1998), Lee et al. (2002), Miró, Casacuberta, Gutiérrez-López, de Landázuri, and Puigdomènech (2000), Smith et al. (2003), Wright et al. (1998)
PDE8	PDE8A PDE8B	cAMP	Airway smooth muscle cells; T-lymphocytes; pulmonary arterial smooth muscle cells; vascular endothelial cells	PF-4957325; dipyridamole;	Glavas et al. (2001), Johnstone et al. (2018), Vang et al. (2010)
PDE9	PDE9A	cGMP	Tracheal smooth muscle cells; pulmonary arterial smooth muscle cells; other cell types largely unknown	BAY-73–6691; PF-04447943;	Patel et al. (2018), Tajima, Shinoda, Urakawa, Shimizu, and Kaneda (2018), Tian et al. (2011)
PDE10	PDE10A	cAMP/cGMP	Pulmonary arterial smooth muscle cells; epithelial cells	Papaverine; TP-10; MP-10 (PF-2545920);	Schmidt et al. (2008), Tian et al. (2011), Wilson et al. (2015), Zhu et al. (2017))
PDE11	PDE11A	cAMP/cGMP	Pulmonary arterial smooth muscle cells; other cell types largely unknown	BC11-38;	Tian et al. (2011)

treated PDE3A-/- and PDE3B-/- mice compared with HDM-treated WT mice. The effect of PDE3 inhibition was further confirmed in HDM-sensitized WT mice using PDE3 inhibitors enoximone and milrinone (Beute et al., 2018), thereby implicating PDE3 as a novel anti-inflammatory target in allergic airway inflammation.

4. PDE4

Four distinct subfamily genes, *Pde4a* to *Pde4d*, encode the cAMP-specific hydrolyzing PDE4 enzyme. The PDE4 family includes a number of splice variants, which share similar and highly conserved catalytic and carboxy terminal domains (Omori & Kotera, 2007). Based on the presence or absence of upstream conserved regions (UCRs) at the amino terminus, PDE4 variants are classified as long forms (which have UCR1 and UCR2 modules), short forms (which lack the UCR1) and super-short forms (which lack UCR1 and have a truncated UCR2) (Omori & Kotera, 2007). It has been demonstrated that UCR1 and UCR2 form a regulatory module that integrates the regulatory effect of phosphorylation by PKA (MacKenzie et al., 2002; Sette & Conti, 1996). In addition, it has been reported that UCR1 and UCR2 play an important role in PDE4 dimerization (Richter & Conti, 2002) and also serve to orchestrate the functional consequences of extracellular signal-related kinase phosphorylation of the PDE4 catalytic domain (MacKenzie, Baillie, McPhee, Bolger, & Houslay, 2000).

The human *Pde4a* gene, for instance, encodes a short isoform called PDE4A1 (Sullivan et al., 1998), and the long forms PDE4A4 (Havekes et al., 2016), PDE4A7 (Johnston et al., 2004), PDE4A10 (Rena et al., 2001) and PDE4A11 (Wallace et al., 2005). PDE4A protein is detected in various tissues, with the PDE4A1 isoform expressed specifically in the cerebellum (Shakur et al., 1995), the PDE4A4 isoform expressed highly in the cerebral cortex and olfactory bulb (McPhee, Pooley, Lobban, Bolger, & Houslay, 1995), the PDE4A8 isoform expressed exclusively in the testis (Bolger, McPhee, & Houslay, 1996), and the PDE4A10 isoform expressed strongly in heart, kidney, olfactory bulb and major island of Calleja (Rena et al., 2001) while the PDE4A11 isoform is expressed predominantly in the stomach, testis, adrenal gland and thyroid (Wallace et al., 2005). Interestingly, a novel human PDE4A8 isoform has been found to be highly expressed in skeletal muscle and brain (Mackenzie et al., 2008). With the exception of PDE4A1, all other PDE4A isoforms have been detected in the lung, especially in inflammatory cells, fibroblasts and pulmonary artery smooth muscle cells (shown in Table 1) (Barber et al., 2004; Mackenzie et al., 2008; Millen, MacLean, & Houslay, 2006; Sachs et al., 2007).

The *Pde4b* family is comprised of a super-short form PDE4B5 (Cheung et al., 2007), a short isoform PDE4B2 (McLaughlin, Cieslinski, Burman, Torphy, & Livi, 1993; Obernolte et al., 1993) and the long isoforms PDE4B1 (Bolger et al., 1993), PDE4B3 (Huston et al., 1997) and PDE4B4 (Shepherd et al., 2003). PDE4B shows ubiquitous expression, and is especially highly detected in the inflammatory cells, the brain and the testis (Cheung et al., 2007). Apart from PDE4B5, which is a brain-specific isoform, other PDE4B isoforms have been detected in various organs and tissues, including the lung (shown in Table 1) (Cheung et al., 2007; Shepherd et al., 2003).

There are seven isoforms of PDE4C, PDE4C1 to PDE4C7 (Engels, Fichtel, & Lübbert, 1994; Engels, Sullivan, Müller, & Lübbert, 1995; Obernolte et al., 1997; Owens et al., 1997). It has been demonstrated that human PDE4C is highly expressed in total brain and particularly in the substantia nigra while it is almost absent in the same regions of rat brain, indicating that PDE4C has a species-specific expression pattern (Engels et al., 1994). In addition, PDE4C is expressed in several different human organs, including but not limited to the brain, liver, lung, kidney and heart. Surprisingly, unlike other PDE4 subfamilies, PDE4C is absent in inflammatory cells (lymphocytes, neutrophils, eosinophils) (Engels et al., 1994, 1995).

The *Pde4d* gene encodes 9 isoforms, PDE4D1 to PDE4D9 (Beavo, Francis, & Houslay, 2006). Six of the PDE4D isoforms (PDE4D3,

PDE4D4, PDE4D5, PDE4D7, PDE4D8 and PDE4D9) are long isoforms (Bolger et al., 1997; Sheppard et al., 2014; Wang et al., 2003) while PDE4D1 and PDE4D2 are short forms (Bolger et al., 1997). In addition, PDE4D6 is categorized as a supershort form with a truncated UCR2 (Wang et al., 2003). The expression of PDE4D is ubiquitous, and different organs, tissues and cells express a varied pattern of PDE4D isoforms which may contribute to the multiple and specialized functions that are unfortunately not yet fully understood (Richter, Jin, & Conti, 2005). In addition, it has been reported that some PDE4D isoforms show a dramatically different tissue distribution pattern in different species. For instance, in humans, PDE4D7 is highly expressed in the lung and kidney, while in the mouse it is expressed in the heart and testis. In the rat, PDE4D7 is expressed in the testis (Wang et al., 2003). Of note is that the mRNA transcripts of all PDE4D isoforms have been detected in the lung, albeit expression levels of PDE4D4 and PDE4D6 are relatively low (Richter et al., 2005).

4.1. PDE4: from basic research to clinical findings

Reports have shown that expression levels of PDE4 isoforms vary between the lung tissue derived from patients with COPD or asthma as compared to those of healthy donors, thereby pointing to PDE4 as an interesting and potential drug target in the treatment of chronic pulmonary diseases. The mRNA expression of PDE4A, PDE4B and PDE4D for example, was significantly augmented in alveolar macrophages from COPD donors compared to that in macrophages from non-smoking controls (Lea, Metryka, Facchinetti, & Singh, 2011). Also, the mRNA of PDE4A4 was significantly increased in alveolar macrophages from smokers with COPD compared to smokers without COPD, suggesting that PDE4A4 could serve as a macrophage-specific anti-inflammatory target in COPD (Barber et al., 2004). Compared to non-smokers, PDE4A4 and PDE4B2 transcripts were significantly up-regulated in peripheral blood monocytes of smokers (Barber et al., 2004). In addition, a significant increase in the mRNA levels of PDE4B and PDE4D, but not of PDE4A or PDE4C, was detected in neutrophils from patients with COPD compared with healthy subjects (Milara et al., 2014). In a genome-wide association study, a novel single nucleotide polymorphism in the PDE4D gene, rs16878037 was identified as being significantly associated with COPD (Yoon et al., 2014).

Cigarette smoke (CS), one of the most important risk factors in COPD (Vogelmeier et al., 2017), plays a critical role in modulating PDE4 subtypes. By using a novel Förster resonance energy transfer based cAMP biosensor in mice *in vivo* and *ex vivo* precision cut lung slices (PCLS), a study was conducted to demonstrate the effect of CS on intracellular cAMP regulation, mainly focusing on cAMP hydrolysis by PDE3 and PDE4 (Zuo et al., 2018). It was shown that CS exposure for 4 days increased the activity of PDE4 in the airway. The upregulation was mainly associated with increased PDE4A (CS *ex vivo* exposure for 24 hours), PDE4B (CS *in vivo* exposure for 4 days) and PDE4D (CS *in vivo* and *ex vivo* exposure) mRNA and protein levels (Zuo et al., 2018). In another study, it was shown that the activity of PDE4 in the lung was higher in mice exposed in utero to CS. In addition, the lung from CS exposed mice exhibited increased PDE4 protein, especially PDE4D5 (Singh et al., 2003, 2009), thereby emphasizing the importance of PDE4D5.

Studies in ASM cells from asthmatic and non-asthmatic patients demonstrated that the production of cAMP induced by the β_2-AR agonist isoproterenol was reduced by about 50%, an effect related to an increased activity of PDEs but not to a change in the expression profile of the β_2-AR (Trian et al., 2011). Further investigation by immunoblots indicated a significant increase of PDE4D in ASM cells from patients with asthma compared to the ones without asthma (Trian et al., 2011). In another study, Jones and colleagues studied the mRNA transcripts of PDE4 subtypes (PDE4A, PDE4B, PDE4C and PDE4D) in CD4+ and CD8+ lymphocytes from healthy and asthmatic subjects (Jones et al., 2007). They found that, although all PDE4 subtypes were present in relatively high quantities in both CD4+ and CD8+ lymphocytes obtained from healthy

and asthmatic subjects, in comparison with healthy subjects no altered mRNA expression level of any PDE4 subtype was detected in mild asthmatic subjects (Jones et al., 2007). These conflicting findings could be partly explained by differences in the cell types. Furthermore, the selection of patients is crucial in these kinds of studies as it may only be possible to detect the molecular differences when studying individuals with a more severe pathology (Jones et al., 2007).

The clinical efficacy and safety of roflumilast has been evaluated in several phase III/IV randomized double-blind clinical trials in the treatment of COPD (shown in Table 2). In all studies, patients were recruited with at least 10-20 years pack history of smoking. Studies M2-124, M2-125, M2-127, M2-128, ACROSS, REACT and RE^{2}SPOND included patients with severe to very severe airflow limitation as assessed by Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (Calverley et al., 2009; Fabbri et al., 2009; Martinez et al., 2015, 2016; Zheng et al., 2014). All clinical studies demonstrated that treatment with 500 μg of roflumilast significantly increased the post-bronchodilator FEV1

value ranging from 39 ml to 80 ml compared with placebo. In patients with frequent exacerbations, roflumilast significantly lowered the rate of exacerbations as compared to placebo (Martinez et al., 2015, 2016). Additionally, roflumilast showed more beneficial effects in patients already receiving treatment with the long-acting β_2-AR agonist salmeterol or the anticholinergic bronchodilator tiotropium (Fabbri et al., 2009) as compared to those that were not, thereby indicating that roflumilast bears the potential to be used as an add-on treatment to the existing therapies in COPD.

4.2. The role of PDE4 inhibition

4.2.1. Anti-inflammatory effect

Due to the fact that PDE4 is widely expressed in inflammatory and immune cells (eosinophils, neutrophils, monocytes, macrophages, T-lymphocytes and B-lymphocytes) (shown in Table 1), it is believed that inhibition of PDE4 is an effective way to reduce the activation and

Table 2
Clinical studies in patients with COPD: focus on roflumilast

Clinical trials	Patients	Study design	Therapy	Key findings	Number of patients with adverse events	References
RECORD	1411 patients (age ≥ 40), history of COPD ≥ 12 months, current or ex-smoker (≥ 1 year of smoking cessation) with a smoking history of ≥ 10 pack-years, PB FEV1 30–80% pred., PB FEV1/FVC ratio ≤ 0.70	Placebo-controlled, double-blind, randomized, multicenter study	Roflumilast 250 μg (n=576), roflumilast 500 μg (n=555), or placebo (n=280) orally once daily for 24 weeks	PB FEV1 was improved significantly with roflumilast 250 μg (by 74 ml) and roflumilast 500 μg (by 97 ml) compared with placebo; health-related quality of life was improved with roflumilast 250 μg and roflumilast 500 μg.	382 (66%) with roflumilast 250 μg, 370 (67%) with roflumilast 500 μg and 174 (62%) in the placebo group	Rabe et al. (2005)
RATIO	1513 patients (age ≥ 40), current or ex-smokers (≥ 1 year of smoking cessation) with a smoking history of ≥ 10 pack-years, PB FEV1 ≤ 50% pred., PB FEV1/FVC ratio ≤ 0.70	Placebo-controlled, double-blind, parallel-group randomized study	Roflumilast 500 μg (n=760) or placebo (n=753) orally once daily for 52 weeks	PB FEV1 increased with roflumilast 500 μg by 39 ml compared with placebo	592 (77.9%) with roflumilast 500 μg, 584 (77.6%) in the placebo group	Calverley et al. (2007)
M2-124 M2-125	3091 patients with COPD (age ≥ 40), with severe airflow limitation, bronchitic symptoms, and a history of exacerbations	Placebo-controlled, double-blind, multicenter study	Roflumilast 500 μg (n=1537) or placebo (n=1554) orally once daily for 52 weeks. Patients were allowed to use SABA or LABA	PB FEV$_1$ was increased with roflumilast 500 μg by 48 ml compared with placebo. The rate of exacerbations that were moderate or severe per patient per year was 1.14 with roflumilast and 1.37 with placebo.	1040 (67%) with roflumilast and 963 (62%) in the placebo group	Calverley et al. (2009)
M2-127	933 patients (age ≥ 40), moderate-to-severe COPD, current or former smokers with a smoking history (≥ 10 pack-years), PB FEV1 40–70% pred., PB FEV1/FVC ratio ≤ 0.70	Double-blind, multicenter study	Roflumilast 500 μg (n=466) or placebo (n=467) orally once daily for 24 weeks, in addition to salmeterol	Roflumilast 500 μg improved mean PB FEV1 by 49 ml in patients treated with salmeterol.	83 (18%) with salmeterol and roflumilast, 14 (3%) with salmeterol and placebo	Fabbri et al. (2009)
M2-128	743 patients (age ≥ 40) with moderate-to-severe COPD, current or former smokers with a smoking history (≥ 10 pack-years), PB FEV1 40–70% pred., PB FEV1/FVC ratio ≤ 0.70	Double-blind, multicenter study	Roflumilast 500 μg (n=371) or placebo (n=372) orally once daily for 24 weeks, in addition to tiotropium	Roflumilast 500 μg improved mean PB FEV1 by 80 ml in those treated with tiotropium.	45 (12%) with tiotropium and roflumilast, and 6 (2%) with tiotropium and placebo	Fabbri et al. (2009)
ACROSS	626 patients with a history of COPD ≥ 12 months, current or ex-smokers with a smoking history (≥ 10 pack-years), ≥ 14 puffs of rescue medication	Placebo-controlled, double-blind, parallel-group, multicenter study	Roflumilast 500 μg (n=313) or placebo (n=313) orally once daily for 24 weeks. Patients were allowed to use ICS + LABA or LAMA	Roflumilast 500 μg improved mean PB FEV1 by 71 ml compared with placebo.	65 (20.6%) with roflumilast and 18 (5.8%) in the placebo group	Zheng et al. (2014)
REACT	1935 patients (age ≥ 40) with a diagnosis of COPD with severe airflow limitation, symptoms of chronic bronchitis, a smoking history (≥ 20 pack-years), at least two exacerbations in the previous year.	Placebo-controlled, double-blind, parallel-group, multicenter study	Roflumilast 500 μg (n=969) or placebo (n=966) orally once daily for 52 weeks together with a fixed ICS and LABA combination	Roflumilast 500 μg lowered the rate of exacerbations by 13.2% according to a Poisson regression analysis and by 14.2% according to a predefined sensitivity analysis using negative binomial regression.	648 (67%) with roflumilast and 572 (59%) in the placebo group	Martinez et al. (2015)
RE^{2}SPOND	2354 patients (age ≥ 40) with severe/very severe COPD, chronic bronchitis, two or more exacerbations and/or hospitalizations in the previous year	Placebo-controlled, double-blind, randomized, multicenter study	Roflumilast 500 μg (n=1178) or placebo (n=1176) orally once daily for 52 weeks, pre-treated with ICS-LABA with or without LAMA for 3 months	Roflumilast 500 μg significantly reduced the rate of moderate or severe exacerbations in a post hoc analysis in patients with a history of more than three exacerbations and/or one or more hospitalizations in the prior year.	804 (68.3%) with roflumilast and 758 (64.6%) in the placebo group	Martinez et al. (2016)

PB, post-bronchodilator; FEV1, forced expiratory volume in 1 second; pred., prediction; FVC, forced vital capacity; ICS, inhaled corticosteroids; SABA, short-acting β_2-adrenoceptor agonists; LABA, long-acting β_2-adrenoceptor agonists; LAMA, long-acting muscarinic antagonists
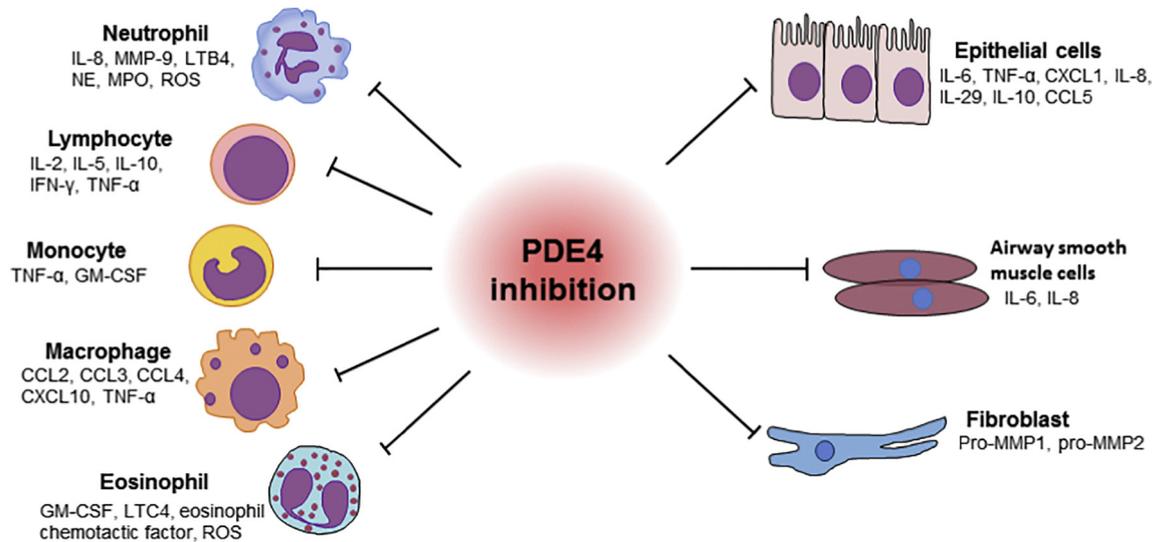
Fig. 2. PDE4 inhibition reduces the release of a variety of pro-inflammatory mediators from key inflammatory cells, including neutrophils, lymphocytes, monocytes, macrophages and eosinophils, as well as structural lung cells, including epithelial cells, airway smooth muscle cells and fibroblasts. IL, interleukin; MMP, matrix metalloproteinase; LTB4, leukotriene B4; NE, neutrophil elastase; MPO, myeloperoxidase; ROS, reactive oxygen species; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor-α; GM-CSF, granulocyte/macrophage colony-stimulating factor; CCL, C-C motif ligand; LTC4, leukotriene C4; CXCL, C-X-C motif ligand.

recruitment of inflammatory cells, and the release of various cytokines (shown in Fig. 2). A range of studies has shown that PDE4 inhibition repressed the release of a variety of pro-inflammatory mediators from neutrophils, such as matrix metalloproteinase (MMP)-9, leukotriene B4, neutrophil elastase, myeloperoxidase and reactive oxygen species (ROS) (Grootendorst et al., 2007; Hatzelmann & Schudt, 2001; Jones, Boswell-Smith, Lever, & Page, 2005; Kubo et al., 2011). Likewise, several research groups showed that PDE4 inhibition was able to block eosinophil infiltration into the lungs (Aoki et al., 2000; Lagente, Pruniaux, Junien, & Moodley, 1995; Silva et al., 2001), to reduce eosinophil survival (Momose et al., 1998), to inhibit degranulation by granulocyte/ macrophage colony-stimulating factor (GM-CSF) or platelet-activating factor (Momose et al., 1998), and to suppress eosinophil chemotaxis, eosinophil cationic protein, CD11b expression and L-selectin shedding (Berends et al., 1997; Grootendorst et al., 2007; Kaneko, Alvarez, Ueki, & Nadel, 1995; Liu et al., 2004). In lung macrophages isolated from peripheral tissues, the PDE4 inhibitor roflumilast and its active metabolite roflumilast N-oxide concentration-dependently decreased the release of chemokine (C-C motif) ligand (CCL)2, CCL3, CCL4, C-X-C motif ligand (CXCL)10 and tumor necrosis factor-α (TNF-α) after stimulation with lipopolysaccharide (LPS) (Buenestado et al., 2012).

In the murine macrophage cell line J774, the PDE4 inhibitor Ro20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone)) showed an inhibitory effect on the oxidant tert-butylhydroperoxide (tBHP)-induced release of tumor necrosis factor- α (TNF-α) protein (Brown et al., 2007). In human monocytes, PDE4 inhibition by rolipram and Ro20-1724 reduced LPS-induced TNF-α and GM-CSF release from monocytes (Seldon, Barnes, Meja, & Giembycz, 1995; Seldon & Giembycz, 2001). In human peripheral CD4+ T cells it has been shown that PDE4 inhibition by RP73401 reduced the release of interleukin (IL)-2, IL-5 and interferon gamma (IFN-γ) (Peter, Jin, Conti, Hatzelmann, & Zitt, 2007). Likewise, house dust mite-stimulated T-cell proliferation was inhibited by PDE4 inhibition (Arp et al., 2003; Manning et al., 1999; Peter et al., 2007). Additionally, PDE4 inhibition reduced cytokine and chemoattractant release from lung structural cells. The PDE4 specific inhibitor rolipram blocked the LPS-induced IL-6 and TNF-α secretion from alveolar epithelial cells (Haddad et al., 2002). Moreover, it was reported that PDE4 inhibitors rolipram and roflumilast decreased LPS-induced CXCL1 release in the bronchial lavage fluid in a C57BL/6 mouse model (Konrad, Bury, Schick, Ngamsri, & Reutershan, 2015). In the same study, rolipram and roflumilast

reduced LPS-induced cytoskeletal remodeling in human distal lung epithelial NCI-H441 cells (Konrad et al., 2015). Furthermore, CHF6001, a highly potent and selective PDE4 inhibitor designed for inhaled administration (Armani et al., 2014; Villetti et al., 2015), reduced rhinovirus (RV1B)-induced IL-8, IL-29, CXCL10 and CCL5 mRNA and protein in human bronchial epithelial BEAS-2b cells (Edwards, Facchinetti, Civelli, Villetti, & Johnston, 2016). In human ASM cells, PDE4 inhibition by RP73401 significantly suppressed the IL-8 release induced by the Toll-like receptor 3 agonist poly I:C (Van Ly et al., 2013). However, in lung fibroblast and human lung microvascular endothelial cells, PDE4 inhibition alone did not effectively decrease the release of inflammatory mediators and other functional molecules but, in combination with appropriate activation of β₂-AR, PDE4 inhibition was able to potently inhibit the inflammatory process (Blease, Burke-Gaffney, & Hellewell, 1998; Tannheimer, Wright, & Salmon, 2012).

In COPD models, the accumulation and infiltration of neutrophils was effectively inhibited by the PDE4 inhibitor cilomilast after 3 days of CS exposure (Leclerc et al., 2006; Martorana, Beume, Lucattelli, Wollin, & Lungarella, 2005). In chronic CS exposure studies, 8 weeks oral administration of the PDE4 inhibitor GPD-1116 markedly attenuated the development of CS-induced emphysema in mice (Mori et al., 2008). Importantly, this finding was confirmed in another study by oral administration of roflumilast for a duration of 7 months, resulting in fully preventing CS-induced emphysema (Martorana et al., 2005). In addition, several different research groups showed that LPS-induced neutrophil recruitment was significantly attenuated by PDE4 inhibition in mouse (McCluskie et al., 2006; Tang et al., 2010), rat (Kubo et al., 2012) and monkey models (Seehase et al., 2012). In patient-related studies, the PDE4 inhibitors roflumilast and cilomilast were able to reduce neutrophil and eosinophil accumulation as well as IL-8, TNF-α and GM-CSF in the sputum of patients with COPD as compared to placebos (Grootendorst et al., 2007; Profita et al., 2003).

In asthma models, the PDE4 inhibitor roflumilast suppressed ovalbumin-induced eosinophil increase in both blood and BAL fluid, and largely reduced the production of IL-4, IL-5, nuclear factor kappa B (NF-κB) and TNF-α (Mokry et al., 2017). These findings were confirmed by using other PDE4 inhibitors, such as rolipram and YM976 (Mokrý et al., 2016; Nejman-Gryz, Grubek-Jaworska, Glapiński, Hoser, & Chazan, 2006). In a separate study it was shown that PDE4B knockout mice had a significant decrease in eosinophil recruitment

and did not develop hyperresponsiveness. More importantly, T(H)2 cytokines (IL-4, IL-5, and IL-13), but not the T(H)1 cytokine IFN-γ, were decreased in the BAL fluid of PDE4B knockout mice, suggesting that PDE4B is a vital target in T(H)2-cell function and in the development of airway hyperresponsiveness in allergic asthma (Jin et al., 2010). Moreover, the PDE4 inhibitor roflumilast significantly suppressed the allergen-induced increase of sputum eosinophils and neutrophils in mild allergic asthma subjects (Gauvreau et al., 2011). Moreover, T-cell receptor-stimulated IFN-γ, IL-2 and IL-17 secretion in BAL fluid was inhibited by PDE4 inhibitors in both mild and moderate asthma patients (Southworth et al., 2018), providing robust evidence for the anti-inflammatory effect of PDE4 inhibitors in asthma patients.

4.2.2. Anti-remodeling effect

Epithelial-to-mesenchymal transition (EMT) is a potential mechanism of small airway remodeling, which contributes to small bronchial narrowing in COPD (Sukhwinder S. Sohal et al., 2010; Sohal & Walters, 2013; Soltani et al., 2010). PDE4 inhibition by roflumilast N-oxide was able to reduce the CS-induced increase in mesenchymal markers (α-smooth muscle actin, vimentin and collagen type I) and the loss in epithelial markers (E-cadherin, ZO-1 and KRT5), to restore CS-induced apoptosis, and to diminish the CS-induced increase in transforming growth factor beta1 (TGF-β1) release as well as phospho ERK1/2 and Smad3 formation, thereby emphasizing PDE4 as a key pharmaceutical target in inhibiting CS-induced EMT (Milara et al., 2014; Milara et al., 2015). Further investigation demonstrated that rolipram or PDE4 small interfering RNA potently inhibited TGF-β1-induced EMT changes in a Smad-independent manner by reducing ROS, p38 and extracellular signal-regulated kinase phosphorylation in the human alveolar epithelial type II cell line A549 (Kolosionek et al., 2009). Additionally, PDE4 inhibition was able to rescue decreased cystic fibrosis transmembrane conductance regulator activity (Blanchard et al., 2014; Lambert et al., 2014; Raju et al., 2017; Schmid et al., 2015), to increase airway surface liquid volume (Schmid et al., 2015; Tyrrell, Qian, Freire, & Tarran, 2015), to stimulate ciliary beating frequency (Milara et al., 2012; Schmid et al., 2015; Zuo et al., 2018), and subsequently to reverse CS-induced mucociliary dysfunction. Also, PDE4 inhibitors roflumilast and piclamilast were able to significantly decrease goblet cell hyperplasia (Kim et al., 2016; Sun et al., 2006).

Furthermore, Sisson and co-workers showed that PDE4 inhibition significantly reduced collagen accumulation, decreased the release of several fibrosis-related chemokines (CCL11, CXCL10, CXCL5 and CCL5), and inhibited fibroblast profibrotic gene expression (type-1 collagen and fibronectin) (Sisson et al., 2018). PDE4 inhibitors were able to attenuate proliferation (Kim et al., 2016; Selige, Hatzelmann, & Dunkern, 2011; Selige, Tenor, Hatzelmann, & Dunkern, 2010; Vecchio et al., 2013) and apoptosis (Park, Ryter, Kyung, Lee, & Jeong, 2013). In lung fibroblasts, RP73-401, a selective PDE4 inhibitor, significantly reduced the MMP-9 activity in ovalbumin-sensitized and -challenged mice (Belleguic et al., 2000). Interestingly, it has been shown that cilomilast and rolipram were able to inhibit fibroblast-mediated collagen contraction (Kohyama et al., 2002; Kohyama et al., 2002). An inhibitory effect of roflumilast on TGF-β-induced fibronectin deposition in human ASM cells and on TGF-β-induced connective tissue growth factor, collagen I and fibronectin protein expression in human bronchial rings was also observed (Burgess et al., 2006). This data point to an anti-remodeling role of PDE4 inhibitors, which would benefit both CODP and asthma.

4.2.3. Bronchodilator effect

In ASM, cAMP regulation is of importance, as elevated cAMP profoundly regulates broncho-relaxation. Since PDE4 is also highly expressed in ASM cells, it is believed that PDE4 inhibitors could also serve as bronchodilators. However, conflicting findings have been reported. It has been proven that roflumilast is able to specifically reduce airway resistance after nebulization in ovalbumin-sensitized guinea pigs, and this finding was further confirmed with a significant decrease

in tracheal and lung smooth muscle contractility after cumulative doses of histamine in the *in vitro* organ bath model (Medvedova et al., 2015). Whilst similar conclusions were made by separate studies which showed that PDE4 inhibition could relax airway tone in isolated bronchial muscle (Schmidt et al., 2000; Shahid et al., 1991), other studies have indicated that PDE4 inhibition alone was not effective (Rabe et al., 1993), especially on allergen- or leukotriene C4-induced contraction of human ASM (Schmidt et al., 2000). Intriguingly, using siRNA targeted to PDE4D5, it has been demonstrated that PDE4D5 plays a vital role in the control of β2-AR-stimulated cAMP levels in human ASM cells (Billington, Le Jeune, Young, & Hall, 2008). The importance of this PDE isoform in modulating contractile ability of ASM was further studied in PDE4D-/- mice. A significant reduction in ASM contractility was observed in isolated PDE4D-/- tracheas, with a dramatic decrease in maximal tension and sensitivity to muscarinic cholinergic agonists (Méhats et al., 2003), thereby indicating that PDE4D was involved in ASM contractility.

5. PDE5

PDE5 is a cGMP-specific hydrolyzing PDE and is comprised of 3 spliced variants, PDE5A1, PDE5A2 and PDE5A3 (Omori & Kotera, 2007). In humans, high PDE5A transcript levels were detected in various tissues, especially in the heart, kidney, lung, skeletal muscle, pancreas and small intestine (Kotera et al., 1999; Yanaka et al., 1998). In the lung, PDE5A is widely expressed in ASM, bronchial epithelial cells, lung fibroblasts, pulmonary vascular smooth muscle of pulmonary arteries as well as in veins and bronchial blood vessels (Aldashev et al., 2005; Dent et al., 1998; Dunkern, Feurstein, Rossi, Sabatini, & Hatzelmann, 2007; Sebkhi, Strange, Phillips, Wharton, & Wilkins, 2003). Currently a series of inhibitors has been designed and is available on the market to target PDE5. These include zaprinast, E4021, dipyridamole, sildenafil, tadalafil, vardenafil, and avanafil (shown in Table 1). While these compounds preferentially inhibit PDE5, none of them is exclusively selective for PDE5, especially at higher concentrations. Intriguingly, most PDE5 inhibitors act excellently as PDE6 inhibitors (Zhang, Feng, & Cote, 2005). Therefore, it is required that more attention is paid to the concentrations of PDE5 inhibitors used in research.

PDE5 has a relatively high expression level in vascular smooth muscle cells. In line with this expression profile, PDE5 inhibitors play a pivotal role in pulmonary hypertension, due to the fact that inhibition of PDE5 results in pulmonary vasodilation and inhibition of vascular hypertrophy and remodeling via the cGMP/PKG signaling pathway (Ghofrani, Osterloh, & Grimminger, 2006). Since asthma and pulmonary hypertension - a common complication of COPD - share several pathological features, such as inflammation, smooth muscle constriction, and smooth muscle cell proliferation, PDE5 may be a potential therapeutic target in the treatment of both asthma and COPD (Chaouat, Naeije, & Weitzenblum, 2008; Said, Hamidi, & Gonzalez Bosc, 2010). Zaprinast, also known as M&B 22948, was originally used as an orally absorbed mast cell stabilizer. Oral administration of 10mg zaprinast was used in 12 patients with asthma induced by histamine or with asthma induced by exercise, respectively. Interestingly, zaprinast had no significant effect on the response to inhaled histamine but a significant effect on the drop in forced expiratory volume in 1s (FEV1) induced by exercise on a treadmill (Rudd, Gellert, Studdy, & Geddes, 1983), indicating that zaprinast could be used in the treatment of exercise-induced asthma.

In addition, it is well established that nitric oxide (NO) released by epithelial ciliated cells, by type II alveolar cells, and by neural fibers, is responsible for ASM cell relaxation (Belvisi, Ward, Mitchell, & Barnes, 1995; Ricciardolo, Sterk, Gaston, & Folkerts, 2004). Several experimental data demonstrated that NO-induced ASM cell relaxation via activation of the soluble guanylyl cyclase resulted in an increase of intracellular cGMP, and the subsequent activation of PKG. Activation of PKG resulted

in an inhibition of the inositol trisphosphate receptor (IP$_3$R), a reduction of Ca^{2+} sensitivity and deactivation of the myosin light-chain kinase, consequently leading to airway relaxation (Perez-Zoghbi, Bai, & Sanderson, 2010). Thus PDE5 inhibitors are likely to induce airway relaxation since PDE5 inhibition is able to contribute to further accumulation of cGMP. In concert with the above findings, therefore, inhibition of PDE5 by zaprinast was able to enhance NO-induced airway relaxation by maintaining high intracellular cGMP concentrations (Perez-Zoghbi et al., 2010). In a separate study, the PDE5 inhibitor tadalafil suppressed acetylcholine and histamine induced contraction in an asthma model of ovalbumin-sensitized guinea pigs (Urbanova et al., 2017). Similar data were obtained in previous studies with sildenafil - a short acting PDE5 inhibitor (Sousa et al., 2011; Toward, Smith, & Broadley, 2004). Additionally, inhibition of PDE5 has proven its effectiveness in inflammation. Intraperitoneal injection of 1.0 mg/kg tadalafil for 7 consecutive days led to a decrease in blood leukocytes and eosinophils, and eosinophils in BAL fluid, confirming findings from several previous studies (Al Qadi-Nassar et al., 2007; Toward et al., 2004; Urbanova et al., 2017). However, even though the concentration of IL-5 was significantly decreased in the tadalafil-treated group compared to the ovalbumin-sensitized group, IL-4 and TNF-α levels in lung homogenates were not significantly suppressed (Urbanova et al., 2017), indicating a plethora of additional complicated mechanisms that may be involved in the potential anti-inflammatory effect of PDE5. Moreover, in patients with severe COPD and modestly increased pulmonary artery pressure, clinical trials with the selective PDE5 inhibitor sildenafil did not improve the gas exchange ability (Blanco et al., 2013), while preventive treatment with tadalafil completely inhibited the development of emphysema, inhibited structural remodeling of the lung vasculature, and alleviated right ventricular systolic pressure as well as right ventricular hypertrophy induced by 6 months CS exposure (Seimetz et al., 2015), thereby indicating additional therapeutic benefits of PDE5 inhibition.

6. PDE7

Since PDE4 is widely distributed in various cell types, oral PDE4 inhibitors inevitably have a limited therapeutic window and are associated with gastrointestinal side effects (Abbott-Banner & Page, 2014). Thus studies of other PDE families are urgently needed for a more targeted therapy. An alternative and promising approach is to inhibit the cAMP-specific PDE isoenzyme PDE7, which is a highly selective cAMP-hydrolyzing PDE (Safavi, Baeeri, & Abdollahi, 2013). Two genes encoding for PDE7, *Pde7a* and *Pde7b*, have been identified in humans (Omori & Kotera, 2007).

There are three isoforms reported in the PDE7A subfamily. The expression of PDE7A1 is ubiquitous and highly detected in the immune system (including spleen, lymph node, blood leukocyte and thymus), whereas PDE7A2 is found mostly in the skeletal muscle, the heart, and the kidney (Bloom & Beavo, 1996; Wang, Wu, Egan, & Billah, 2000). It has been demonstrated that PDE7A3 is mainly expressed in the immune system, the heart, skeletal muscle and the testis (Glavas, Ostenson, Schaefer, Vasta, & Beavo, 2001; Omori & Kotera, 2007). PDE7B1 is the only PDE7B isoform that has been identified in humans. However, there are three splice variants, PDE7B1 to PDE7B3, in rats (Omori & Kotera, 2007). PDE7B which has approximately 70% homology to PDE7A is detected in a variety of tissues, such as liver, brain, heart and skeletal muscle (Gardner, Robas, Cawkill, & Fidock, 2000; Sasaki, Kotera, Yuasa, & Omori, 2000; Strahm, Rane, & Ekström, 2014). In the lung, PDE7A1, PDE7A2 and PDE7A3 are expressed in T cells, in the airways as well as in vascular structural cells, with PDE7B exhibiting a lower distribution (Smith et al., 2003).

PDE7 is considered to be a promising anti-inflammatory target for alleviating chronic inflammation since PDE7 exists ubiquitously in pro-inflammatory and immune cells (Giembycz & Smith, 2006; Smith et al., 2003), albeit no significant differences were observed in the

mRNA expression of PDE7A and PDE7B between healthy and mild asthmatic or COPD subjects (Jones et al., 2007). It has been shown that T-lymphocyte activation up-regulated the mRNA and protein expression of both PDE7A1 and PDE7A3 (Glavas et al., 2001). Moreover, inhibition of PDE7 expression using PDE7 antisense oligonucleotides was able to dramatically decrease human T-lymphocyte proliferation in a PKA-dependent manner, indicating that PDE7 plays an essential role in T-lymphocyte activation (Li, Yee, & Beavo, 1999). A similar conclusion was drawn by using the PDE inhibitor T-2585 in a dose range (0.1–10μm) at which the drug inhibits PDE7A activity. The study showed that PDE7A inhibition could suppress IL-2, IL-4 and IL-5 mRNA expression and cell proliferation of human peripheral T-lymphocytes (Nakata et al., 2002). In contrast to these data obtained in humans, Yang and colleagues reported completely different findings using PDE7A-deficient mouse in which the deletion of the PDE7A gene did not exhibit any reduction in terms of *in vitro* T-lymphocyte proliferation and cytokine production (IL-2, IFN-γ, or TNF-α) (Yang et al., 2003). Moreover, no significant improvement of airway inflammation and airway hyperreactivity could be observed in ovalbumin-sensitized mice using the PDE7 specific inhibitor compound 21a (Chevalier et al., 2012). These studies point to different regulatory mechanisms of PDE7 on cAMP signaling in humans and mice.

In addition, several selective small-molecule PDE7 inhibitors have been reported and used in *in vivo* and *in vitro* studies (Kadoshima-Yamaoka et al., 2009; Martín-Álvarez et al., 2017; Safavi et al., 2013; Smith et al., 2004). The sulfonamide PDE7 inhibitor BRL 50481 is significantly more active against PDE7A than against PDE7B (IC50: PDE7A 0.15 μM, PDE7B 12.1 μM) (Alaamery et al., 2010). It was shown that BRL 50481 was able to enhance the inhibitory effect of the PDE4 inhibitor rolipram on the TNF-α release from blood monocytes and lung macrophages, even though the inhibitory effect of BRL 50481 alone was very limited, indicating that BRL 50481 acted additively with other PDE inhibitors to inhibit pro-inflammatory cells (Smith et al., 2004). Additionally, a novel series of benzyl derivatives of 2,1,3-benzo- and benzothieno [3,2-a] thiadiazine 2,2-dioxides (Castro, Abasolo, Gil, Segarra, & Martinez, 2001; Martínez et al., 2000), 5-substituted 8-chloro-spirocyclohexane-quinazolinones (Bernardelli et al., 2004), thiadiazoles (Vergne et al., 2004) and thioxoquinazoline derivatives (Castaño et al., 2009) have been developed as potent and selective PDE7 inhibitors. Their therapeutic effects have been demonstrated in neurological disorders, for instance Parkinson disease (Banerjee et al., 2012; Morales-Garcia et al., 2011), Alzheimer's disease (Perez-Gonzalez et al., 2013; Pérez-Torres et al., 2003), spinal cord injury (Paterniti et al., 2011), autoimmune encephalomyelitis (Martín-Álvarez et al., 2017) as well as multiple sclerosis (Mestre et al., 2015). However, their pharmacological effects have not been investigated in pulmonary disorders, including asthma and COPD. Therefore, more studies are urgently needed to explore the potential therapeutic effects of novel PDE7 inhibitors in pulmonary disorders.

7. PDE8

As another cAMP-specific hydrolyzing PDE, PDE8, consisting of PDE8A and PDE8B, exhibits a higher-affinity and lower K_m ($\approx$0.04 - 0.15 μM) for cAMP compared to other PDE isoforms, thus acting as a potential drug target to shape low-level intracellular cAMP signals (Fisher, Smith, Pillar, St Denis, & Cheng, 1998; Hayashi et al., 1998; Soderling, Bayuga, & Beavo, 1998; Vang et al., 2010; Yan, Wang, Cai, & Ke, 2009). PDE8A is highly expressed in the testis, liver and heart (Fisher et al., 1998; Soderling et al., 1998), whereas PDE8B is richly found in the thyroid and brain (Hayashi et al., 1998). In the lung, both PDE8 isoforms have been detected, albeit the relevant expression levels are low. As PDE8 is one of the PDEs that cannot be inhibited by the non-selective PDE inhibitor IBMX, there is urgent need to design and develop new PDE8 selective inhibitors to explore the physiological and pathological role of PDE8 (Soderling et al., 1998; Soderling & Beavo, 2000). So far,

only a few PDE8 inhibitors are available on the market and two out of three are dual PDE inhibitors (dipyridamole, PDE5/8; BC8-15, PDE4/8). The recently developed PDE8 selective inhibitor PF-4957325 by Pfizer has been widely used in PDE8 research. This novel compound has an IC_{50} value of 0.7 nM for PDE8A, < 0.3 nM for PDE8B and >1.5 µM for other PDE isoforms (Vang et al., 2010).

It has been reported that PDE8 plays a vital role in adrenal steroidogenesis (Shimizu-Albergine, Tsai, Patrucco, & Beavo, 2012; Tsai, Shimizu-Albergine, & Beavo, 2011), Ca^{2+} movement in ventricular myocytes (Patrucco, Albergine, Santana, & Beavo, 2010), and thyroid dysfunction (Gamanuma et al., 2003). In the lung, Johnstone and colleagues demonstrated for the first time that PDE8A was highly expressed in human ASM cells and that inhibition of PDE8, together with β_2-AR stimulation by isoproterenol, profoundly reduced serum-induced human ASM cell proliferation compared to isoproterenol alone (Johnstone et al., 2018), thereby indicating a potential pharmaceutical benefit of PDE8 in ASM cells.

In addition, T cell activation up-regulated both mRNA and protein expression of PDE8A1, suggesting a potential therapeutic role of PDE8 in immune cells (Glavas et al., 2001). Since lymphocyte migration is a key feature in inflammatory diseases, such as COPD and asthma, inhibition of the migration of activated lymphocytes would therefore provide a full therapeutic effect (Ainslie, McNulty, Huynh, Symon, & Wardlaw, 2002). It was reported that PDE8 was able to inhibit the migration of unstimulated and concanavalin A-stimulated mouse splenocytes. This inhibition was further increased by forskolin and diminished by the PKA antagonist Rp-cAMPS, indicating that PDE8 may act as a promising novel target for inhibition of chemotaxis of activated lymphocytes (Dong, Osmanova, Epstein, & Brocke, 2006). In addition, T cell interaction with vascular endothelial cells plays a crucial role during the inflammatory process (Carman & Martinelli, 2015). In spite of the abundant expression of PDE3 and PDE4 in T lymphocytes, the highly selective PDE4 inhibitor RP73401 and the PDE3-selective inhibitor motapizone failed to reduce T cell adhesion to endothelial cells, whereas inhibition of PDE8 by dipyridamole suppressed adhesion and directed migration of activated T cells (Vang et al., 2010). Dipyridamole also modulated the gene expression of recruitment chemokine CXCL12 and vascular adhesion molecules (vascular cell adhesion protein 1, intercellular adhesion molecule 1, and tight junction molecule claudin-5), indicating that PDE8 might serve as a novel and promising target for inhibition of activated T-lymphocyte migration from the bloodstream into the tissue during the inflammatory response (Vang et al., 2010).

8. Dual PDE inhibitors

Although the orally administered PDE4 selective inhibitor roflumilast N-oxide has been approved by both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) to be used as an add-on treatment for severe COPD patients associated with bronchitis and a history of frequent exacerbations (Vogelmeier et al., 2017), unwanted side effects including nausea, headache and gastrointestinal issues have been reported, thereby representing a major drawback for the wider therapeutic use of PDE4 inhibitors (Page & Spina, 2012). Therefore, it is conceivable that administration of PDE4 inhibitors together with another PDE family inhibitor via inhalation at a concentration that does not cause any side effects could provide an additive or even synergistic therapeutic benefit (Giembycz, 2005; Turner et al., 2016).

8.1. Dual PDE 3/4 inhibitors

Due to the wide distribution of PDE3 and PDE4 in the lung structural cells and most inflammatory cells, dual inhibition of PDE3/4 appears to be an attractive way to target pathological key characteristics of COPD, particularly as one might expect additive anti-inflammatory and

bronchodilator effects. Milara et al. reported that inhibition of PDE3 with the PDE3 selective inhibitor motapizone alone or inhibition of PDE4 with the PDE4 selective inhibitor rolipram alone resulted in about 20% reduction of LPS-induced IL-8 and TNF-α secretion from human alveolar macrophages, whereas combined PDE3/4 inhibition caused an up to 90% reduction of LPS-induced cytokine secretion (Milara et al., 2011). Moreover, oxidative stress induced by H_2O_2 and CS, which is known to profoundly minimize inhibitory effects of corticosteroids, did not impair the inhibitory effect of PDE3/4 inhibition (Milara et al., 2011). The synergistic anti-inflammatory effect of combined PDE3/4 inhibition was also confirmed in other studies (Hatzelmann & Schudt, 2001; Rieder et al., 2013). In addition, a greater effect on glucocorticoid- and β_2-AR agonist-dependent gene transcription was observed upon combined PDE3/4 inhibition compared to when either a PDE3 or PDE4 inhibitor was used alone (BinMahfouz et al., 2015; Giembycz & Newton, 2011), suggesting that dual PDE3/4 inhibition may play an add-on role to long-acting β_2-AR agonists and inhaled corticosteroid plus long-acting β_2-AR agonist combinations, further enhancing their therapeutic efficacies (Giembycz & Maurice, 2014). As regards the potential bronchodilator effects of PDE3/4 inhibition, it has been demonstrated that the combination could significantly relax the inherent bronchial tone (Calzetta et al., 2013; Rabe et al., 1993).

Many dual PDE3/4 inhibitors have been tested at the pre-clinical stage. At least five dual inhibitors have reached the clinical trial stage, including zardaverine, benzafentrine, pumafentrine, tolafentrine and RPL554 (Page, 2014). It was reported that inhalation of zardaverine led to a significant increase of FEV1 and specific airway conductance within the first hour of application to patients with reversible bronchial obstruction compared to placebo (Brunnée, Engelstätter, Steinijans, & Kunkel, 1992). However, three patients out of twelve reported side effects (headache, drowsiness, vertigo, nausea), and one patient dropped out of the study due to vomiting (Brunnée et al., 1992). Another phase II clinical trial in ten patients with partially reversible chronic airflow obstruction reported that single doses of 1.5 mg, 3.0 mg, or 6.0 mg zardaverine by metered dose inhaler did not improve airway functions compared to 0.3 mg salbutamol and placebo (Ukena, Rentz, Reiber, & Sybrecht, 1995). The potential bronchodilator effect of another dual PDE3/4 inhibitor, benzafentrine, was examined in healthy volunteers by the oral, intravenous, and inhalation routes (Foster, Rakshi, Carpenter, & Small, 1992). Oral administration of 9 mg, 20 mg, or 90 mg benzafentrine failed to induce any bronchodilator response. However, intravenous administration of 20 mg or 40 mg showed a short-lived bronchodilator response without affecting the blood pressure or pulse rate. Benzafentrine produced the most significant bronchodilator effect upon application via inhalation, leading to a dose-dependent broncho-protection to challenge with methacholine, with an effective dose (ED50) of approximately 9.2 mg (Foster et al., 1992). Except for RPL554, all other dual inhibitors have not been developed beyond the clinical stage due to unwanted side effects on the gastrointestinal system.

RPL554, as one of the most effective dual inhibitors, was shown to relax bronchial AMS in both the guinea pig model and in isolated human (medium and small) bronchi. It was also shown to increase cilia beat frequency and mucociliary clearance in human primary bronchial epithelial cells by activation of the cystic fibrosis transmembrane conductance regulator gene, by inhibition of TNF-α release from LPS-stimulated human monocytes and by suppression of monocyte proliferation, which attests to its bronchodilator and anti-inflammatory effects (Boswell-Smith et al., 2006; Calzetta et al., 2015; Turner et al., 2016; Venkatasamy & Spina, 2016). Additionally, oral administration of 10 mg/kg RPL554 1 hour before ovalbumin challenge in guinea pigs induced a significant reduction of eosinophil infiltration into the lung. A similar effect was observed by RPL554 inhalation in conscious guinea pigs 1.5 h before ovalbumin exposure (Boswell-Smith et al., 2006). Franciosi et al. demonstrated that 0.018 mg/kg RPL554 inhalation

produced bronchodilation with a 17.2% increase of maximum FEV1 in mild-to-moderate COPD patients compared to placebo. Moreover, in healthy volunteers, the percentage of neutrophils in sputum after 6 hours LPS challenge was not significantly changed after 0.018 mg/kg RPL554 inhalation. It was shown, however, that RPL554 significantly reduced the absolute numbers of total cells - neutrophils, macrophages, lymphocytes, and eosinophils - in sputum (Franciosi et al., 2013), suggesting that the molecule also possessed substantial anti-inflammatory activity. More importantly, the inhaled dose of RPL554 was well tolerated by both healthy volunteers and patients without gastrointestinal or cardiac side effects (Franciosi et al., 2013). Of note, however, is that only short-term inhalation of RPL554 for up to 7 days was monitored in the study of Franciosi et al. (Wedzicha, 2013), and therefore the long-term therapeutic and cardiovascular side effects of RPL554 need to be carefully assessed in future studies.

Although RPL554 is considered as a dual PDE3/4 inhibitor, it is speculated based on its 3000-fold higher affinity to PDE3 compared to PDE4 that RPL554 acts primarily as a PDE3 inhibitor rather than as a dual PDE3/4 inhibitor (IC50 for PDE3: 0.4 nM; PDE4: 1479 nM) (Boswell-Smith et al., 2006). In that regard, it is likely that the clinical benefits in human subjects are due to PDE3 inhibition rather than to dual PDE3/4 inhibition. Therefore, more investigation is needed to explore the real pharmaceutical target of RPL554. Also, novel dual PDE3/4 inhibitors with similar inhibitory potencies on PDE3 and PDE4 are necessary to test in future studies.

8.2. Dual PDE 4/7 inhibitors

PDE7 is another leading candidate in the dual inhibitor family approach because of its anti-inflammatory ability (Li et al., 1999; Nakata et al., 2002). Several groups studied the possibility of inhibiting both PDE4 and PDE7. In normal human bronchial epithelial cells, cytokine (TNF-α, IL-1β and IFN-γ)-induced secretion of IL-8 and human monocyte chemoattractant protein-1 was significantly decreased to baseline levels by using multi-target antisense oligonucleotides to address specifically PDE4B/4D and 7A protein expression (Fortin et al., 2009). In addition, the multi-target antisense oligonucleotides showed promising protection against the CS-induced recruitment of neutrophils, keratinocyte chemoattractant production and pro-MMP-9 upregulation (Fortin et al., 2009), thereby indicating a potent and broad anti-inflammatory effect against CS-induced lung inflammation. Additionally, Mokry and colleagues reported on the relaxing effect of combined PDE4/7 inhibition (rolipram plus BRL50481) on acetylcholine-induced lung and airway contraction in ovalbumin-sensitized guinea pigs (Mokry, Joskova, Mokra, Christensen, & Nosalova, 2013). In another study, BC54, a novel dual PDE4/7 inhibitor, showed a superior anti-inflammatory effect on TNF-α production by macrophages and IL-2 production by T-lymphocytes as compared to rolipram alone or to a combination of rolipram and BRL50481 (de Medeiros et al., 2017). However, there is no further clinical evidence to prove the superior anti-inflammatory activity of dual PDE4/7 inhibition over PDE4 inhibition alone, therefore, more studies are needed (Giembycz & Maurice, 2014).

8.3. Dual PDE 4/5 inhibitors

Increasing the intracellular levels of cAMP and cGMP via PDE4 and PDE5 inhibition, respectively, is an attractive idea as a novel treatment in respiratory diseases. Intraperitoneal treatment with either roflumilast (daily dose 1.0 mg/kg body weight) or tadalafil (daily dose 1.0 mg/kg body weight) for 7 days reduced the airway resistance after nebulization of histamine, decreased airway contraction to cumulative doses of histamine and acetylcholine, and suppressed the production of several inflammatory mediators (IL-4, IL-5, NF-κB, and TNF-α) in ovalbumin-sensitized guinea pigs. However, the combination of roflumilast and tadalafil at a reduced dose (daily dose of 0.5 mg/kg

body weight) did not show any additive effect compared to PDE4 inhibition alone (Mokry et al., 2017).

9. Future directions

PDEs are attractive pharmaceutical targets for COPD and asthma treatment as their inhibition is able to induce broad anti-inflammatory and/or bronchodilator effects (Chung, 2006; Giembycz & Maurice, 2014). More importantly, dual inhibition of PDE3/4 by inhalation maximizes the therapeutic potential of the inhibitors, and minimizes the unwanted side effects (BinMahfouz et al., 2015). However, considering that PDE is composed of at least 21 different isoforms, the key challenge is to develop PDE isoform-selective inhibitors, which could be used to study the potential inhibitory roles during the pathogenesis of COPD and asthma.

Even though the oral administration of the PDE4 inhibitor roflumilast has been approved for the treatment of severe COPD patients associated with bronchitis and a history of frequent exacerbations, unwanted side effects including nausea and vomiting still limit the oral administration of PDE4 inhibitors (Giembycz & Maurice, 2014). As inhalation delivers the drugs directly to the site of action, it is likely to assume that this administration route may improve the therapeutic index required to overcome the unwanted side effects. However, to date none of the very potent inhaled PDE4 inhibitors have shown any convincing evidence of efficacy in the treatment of respiratory diseases (D. Singh et al., 2016; Watz, Mistry, Lazaar, & IPC101939 investigators, 2013). GSK256066 is an inhaled PDE4 inhibitor developed by GlaxoSmithKline to treat patients with COPD. In a phase IIa, multi-center, parallel-group, double-blind, three-arm, placebo-controlled, four-week, randomized study, two doses (25 μg, 87.5 μg) of GSK256066 were tested in patients with moderate COPD (Watz et al., 2013). Although there was an increase in post-bronchodilator FEV1 at both GSK256066 concentrations being applied compared to placebo on day 28, these differences were not statistically significant. Additionally, no changes were observed in the relative proportion or total numbers of neutrophils or macrophages in the sputum of treated subjects (Watz et al., 2013). Another inhaled PDE4 inhibitor is CHF6001 developed by Chiesi Farmaceutici. It was reported that CHF6001 was well tolerated when administered by once daily single-dose (100 μg, 300 μg, 600 μg, 1200 μg, 1600 μg) dry-powder inhalation for 7 days (Mariotti, Govoni, Lucci, Santoro, & Nandeuil, 2018). In a double blind, placebo controlled, 3-way cross-over study, 36 atopic asthmatics (not under treatment with inhaled corticosteroids and characterized by a late asthmatic response) received CHF6001 400 μg or 1200 μg or placebo once a day using a dry powder inhaler for 9 days. Allergen challenges were performed on day 9 and induced sputum was obtained 10 hours after challenge (Singh et al., 2016). Both CHF6001 doses significantly increased FEV1, while the difference between the two doses was not significant. CHF6001 caused a greater reduction in sputum eosinophil counts as compared to placebo, albeit no significance was observed (Singh et al., 2016). Taken together, based on the current knowledge, the addition of inhaled/oral administration of PDE4 inhibitors seems to exert beneficial effects for COPD patients but obviously more clinical trials are warranted to strengthen the initial findings.

Air pollution-induced oxidative stress is another important risk factor in the pathogenesis of COPD and asthma (Bernardo, Bozinovski, & Vlahos, 2015; Holguin, 2013; Kirkham & Rahman, 2006; Wang et al., 2018). Increased attention has been focused specifically on diesel exhaust exposure (Hart, Eisen, & Laden, 2012; Hart, Laden, Schenker, & Garshick, 2006; Wade & Newman, 1993). During diesel fuel combustion, several types of pollutants are released, including but not limited to particulate matter, metals and polycyclic aromatic hydrocarbons (PAH) (Steiner, Bisig, Petri-Fink, & Rothen-Rutishauser, 2016). It has been proven that diesel exhaust is highly associated with lung inflammation (de Brito et al., 2018; De Grove et al., 2018; Steiner et al., 2016). Moreover, a few studies reported that PAH exposure reduced
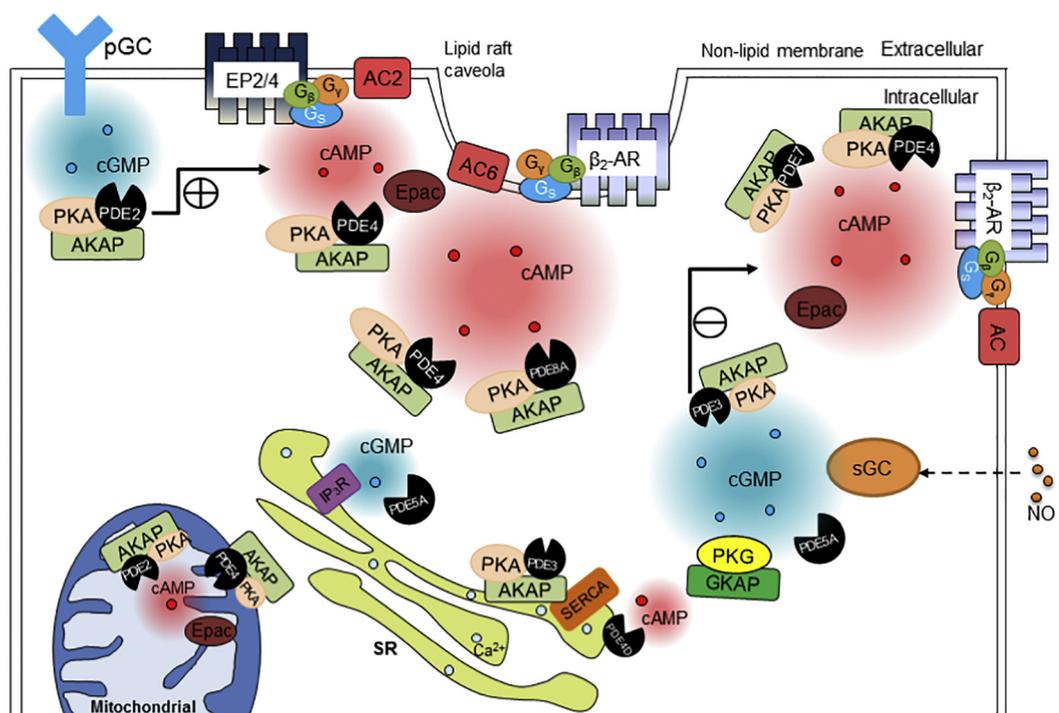
Fig. 3. Schematic overview of cyclic nucleotide compartmentalization in the lung. PDEs dynamically control cAMP and cGMP signals in different subcellular microdomains. Consequently, the activities of downstream effectors, such as PKA, Epacs and PKG, are modulated. PDEs are subject to highly spatio-temporal dynamics, meaning individual PDEs are most likely to be recruited to specific locations at specific time points based on different stimulations/activations. A-kinase anchoring proteins (AKAPs) are a group of scaffolding proteins with the ability to associate with PKA via a short α-helical structure. It is known that some PDEs and AKAPs are highly expressed, for instance, in mitochondria (AKAP1) or at the plasma membrane (AKAP5 and AKAP12) (Cong et al., 2001; Merrill & Strack, 2014; Tao & Malbon, 2008). However, the molecular link between AKAPs and PDEs has not yet been studied in specific compartments in the lung. PDEs, phosphodiesterases; PKA, cAMP-dependent protein kinase A; Epacs, exchange proteins directly activated by cAMP; PKG, cGMP-dependent protein kinase G; AKAPs, A-kinase anchoring proteins; EP, E prostanoid receptor; β_2-AR, β_2-adrenoceptor; AC, adenylyl cyclase; pGC, particulate guanylate cyclase; sGC, soluble guanylyl cyclase; NO, nitric oxide; IP3R, inositol trisphosphate receptor; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

cAMP production induced by the β_2-AR agonist procaterol in primary murine tracheal epithelial cells and human ASM cells, thereby indicating that the cAMP signaling pathway is impaired by PAH (Factor et al., 2011). Therefore, further investigation is needed to study the effect of air pollution, including diesel fuel, on cyclic nucleotide signaling.

Cyclic nucleotides, as the most ubiquitous second messengers, control a wide range of physiological and pathophysiological processes by modulating signaling cascades in a spatio-temporal manner. Comprehensive understanding of the fluctuations (generation and degradation) of cAMP and cGMP and their potential functions within certain compartments will most likely help in the screening of novel pharmaceutical targets which have higher efficacy and less side effects (shown in Fig. 3). Recently, Johnstone and colleagues used classical molecular biological tools to study the role of PDE8 in β_2-AR-AC6 and E prostanoid receptor (EP)2/4-AC2 compartments (Johnstone et al., 2018). It was demonstrated that knockdown of PDE8A using shRNA evoked more cAMP production in response to forskolin and 3-isobutyl-1-methylxanthine specifically in AC6 overexpressing human ASM cells, but not in AC2 overexpressing ASM cells, indicating that β_2-AR/AC6/PDE8 is a functional signalosome. Also, they found that β_2-AR/AC6/PDE8 are mainly expressed in caveolae (Johnstone et al., 2018) (shown in Fig. 3). This finding emphasizes the microdomain-specific cAMP modulation, which helps to fully understand cAMP and cGMP functions as second messengers. However, of note, it is difficult to monitor intracellular cAMP dynamics using standard biochemical techniques. So far, several FRET based biosensors have been developed to achieve real-time visualization of cAMP and cGMP with high spatial and temporal resolutions (Nikolaev, Bünemann, Hein, Hannawacker, & Lohse, 2004; Pavlaki & Nikolaev, 2018; Sprenger et al., 2015; Violin et al., 2008). It was reported by Billington and colleagues that the cAMP biosensor CFP-Epac (dDEP,CD)-VENUS could be used to study the β-AR-mediated signaling kinetics in human primary ASM cells,

revealing ligand and dose dependent differences of several β-AR agonists (indacaterol, isoproterenol, salmeterol and formoterol) (Billington & Hall, 2011). Using another cAMP biosensor with fluorescently tagged PKA subunits, Schmid et al. studied the effect of CS on the PDE4 inhibitor roflumilast-induced intracellular cAMP changes in fully differentiated normal human bronchial epithelial cells (Schmid et al., 2015). Recently, a study monitored intracellular cAMP dynamics in the airway using PCLS and cAMP reporter Epac1-camps mice, indicating the possibility to visualize cAMP fluctuations in intact lung tissue (Zuo et al., 2018). It is noteworthy that all of these studies used globally expressed cAMP biosensors in order to study cytosolic cAMP changes. However, cAMP localizes to specific subcellular microdomains, therefore, using microdomain-specific targeted cAMP biosensors to study the local cAMP dynamics will be more helpful to reveal microdomain-specific cAMP dynamics (Musheshe, Schmidt, & Zaccolo, 2018; Sprenger & Nikolaev, 2013). In addition, there are no reports using cGMP FRET biosensors to investigate the intracellular cGMP levels in either lung structural cells or tissues so far. Thus, it is conceivable that monitoring microdomain-specific intracellular cAMP and cGMP levels and, more importantly, their crosstalk modulated by PDE2 and PDE3 will provide important new knowledge that will help to design novel drugs targeting cyclic nucleotides with higher efficacy and with less side effects.

Author contributions

H.Z., I.C., N.M., V.O.N. and M.S. wrote the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

References

Abbott-Banner, K. H., & Page, C. P. (2014). Dual PDE3/4 and PDE4 inhibitors: novel treatments for COPD and other inflammatory airway diseases. *Basic & Clinical Pharmacology & Toxicology 114*(5), 365–376. https://doi.org/10.1111/bcpt.12209.

Ainslie, M. P., McNulty, C. A., Huynh, T., Symon, F. A., & Wardlaw, A. J. (2002). Characterisation of adhesion receptors mediating lymphocyte adhesion to bronchial endothelium provides evidence for a distinct lung homing pathway. *Thorax 57*(12), 1054–1059.

Al Qadi-Nassar, B., Bichon-Laurent, F., Portet, K., Tramini, P., Arnoux, B., & Michel, A. (2007). Effects of L-arginine and phosphodiesterase-5 inhibitor, sildenafil, on inflammation and airway responsiveness of sensitized BP2 mice. *Fundamental & Clinical Pharmacology 21*(6), 611–620. https://doi.org/10.1111/j.1472-8206.2007.00523.x.

Alaamery, M. A., Wyman, A. R., Ivey, F. D., Allain, C., Demirbas, D., Wang, L., ... Hoffman, C. S. (2010). New classes of PDE7 inhibitors identified by a fission yeast-based HTS. *Journal of Biomolecular Screening 15*(4), 359–367. https://doi.org/10.1177/1087057110362100.

Aldashev, A. A., Kojonazarov, B. K., Amatov, T. A., Sooronbaev, T. M., Mirrakhimov, M. M., Morrell, N. W., ... Wilkins, M. R. (2005). Phosphodiesterase type 5 and high altitude pulmonary hypertension. *Thorax 60*(8), 683–687. https://doi.org/10.1136/thx.2005.041954.

Aoki, M., Fukunaga, M., Kitagawa, M., Hayashi, K., Morokata, T., Ishikawa, G., ... Yamada, T. (2000). Effect of a novel anti-inflammatory compound, YM976, on antigen-induced eosinophil infiltration into the lungs in rats, mice, and ferrets. *The Journal of Pharmacology and Experimental Therapeutics 295*(3), 1149–1155.

Armani, E., Amari, G., Rizzi, A., De Fanti, R., Ghidini, E., Capaldi, C., ... Villetti, G. (2014). Novel class of benzoic acid ester derivatives as potent PDE4 inhibitors for inhaled administration in the treatment of respiratory diseases. *Journal of Medicinal Chemistry 57*(3), 793–816. https://doi.org/10.1021/jm401549m.

Arp, J., Kirchhof, M. G., Baroja, M. L., Nazarian, S. H., Chau, T. A., Strathdee, C. A., ... Madrenas, J. (2003). Regulation of T-cell activation by phosphodiesterase 4b2 requires its dynamic redistribution during immunological synapse formation. *Molecular and Cellular Biology 23*(22), 8042–8057. https://doi.org/10.1128/MCB.23.22.8042-8057.2003.

Banerjee, A., Patil, S., Pawar, M. Y., Gullapalli, S., Gupta, P. K., Gandhi, M. N., ... Gharat, L. A. (2012). Imidazopyridazinones as novel PDE7 inhibitors: SAR and in vivo studies in Parkinson's disease model. *Bioorganic & Medicinal Chemistry Letters 22*(19), 6286–6291. https://doi.org/10.1016/j.bmcl.2012.07.077.

Barber, R., Baillie, G. S., Bergmann, R., Shepherd, M. C., Sepper, R., Houslay, M. D., & Heeke, G. V. (2004). Differential expression of PDE4 cAMP phosphodiesterase isoforms in inflammatory cells of smokers with COPD, smokers without COPD, and nonsmokers. *American Journal of Physiology. Lung Cellular and Molecular Physiology 287*(2), L332–L343. https://doi.org/10.1152/ajplung.00384.2003.

Beavo, J. A., & Brunton, L. L. (2002). Cyclic nucleotide research — still expanding after half a century. *Nature Reviews Molecular Cell Biology 3*(9), 710–718. https://doi.org/10.1038/nrm911.

Beavo, J. A., Francis, S. H., & Houslay, M. D. (2006). Cyclic nucleotide phosphodiesterases in health and disease. Retrieved from https://www.crcpress.com/Cyclic-Nucleotide-Phosphodiesterases-in-Health-and-Disease/Beavo-Francis-Houslay/p/book/9780849396687.

Beghè, B., Rabe, K. F., & Fabbri, L. M. (2013). Phosphodiesterase-4 inhibitor therapy for lung diseases. *American Journal of Respiratory and Critical Care Medicine 188*(3), 271–278. https://doi.org/10.1164/rccm.201301-0021PP.

Belleguic, C., Corbel, M., Germain, N., Boichot, E., Delaval, P., & Lagente, V. (2000). Reduction of matrix metalloproteinase-9 activity by the selective phosphodiesterase 4 inhibitor, RP 73-401 in sensitized mice. *European Journal of Pharmacology 404*(3), 369–373.

Belvisi, M. G., Ward, J. K., Mitchell, J. A., & Barnes, P. J. (1995). Nitric oxide as a neurotransmitter in human airways. *Archives Internationales De Pharmacodynamie Et De Therapie 329*(1), 97–110.

Berends, C., Dijkhuizen, B., de Monchy, J. G., Dubois, A. E., Gerritsen, J., & Kauffman, H. F. (1997). Inhibition of PAF-induced expression of CD11b and shedding of L-selectin on human neutrophils and eosinophils by the type IV selective PDE inhibitor, rolipram. *The European Respiratory Journal 10*(5), 1000–1007.

Bernardelli, P., Lorthiois, E., Vergne, F., Oliveira, C., Mafroud, A. -K., Proust, E., ... Li, M. (2004). Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 2: Optimization of 5,8-disubstituted derivatives. *Bioorganic & Medicinal Chemistry Letters 14*(18), 4627–4631. https://doi.org/10.1016/j.bmcl.2004.07.010.

Bernardo, I., Bozinovski, S., & Vlahos, R. (2015). Targeting oxidant-dependent mechanisms for the treatment of COPD and its comorbidities. *Pharmacology & Therapeutics 155*, 60–79. https://doi.org/10.1016/j.pharmthera.2015.08.005.

Bernareggi, M. M., Belvisi, M. G., Patel, H., Barnes, P. J., & Giembycz, M. A. (1999). Antispasmogenic activity of isoenzyme-selective phosphodiesterase inhibitors in guinea-pig trachealis. *British Journal of Pharmacology 128*(2), 327–336. https://doi.org/10.1038/sj.bjp.0702779.

Beute, J., Lukkes, M., Koekoek, E. P., Nastiti, H., Ganesh, K., de Bruijn, M. J., ... KleinJan, A. (2018). A pathophysiological role of PDE3 in allergic airway inflammation. *JCI Insight 3*(2). https://doi.org/10.1172/jci.insight.94888.

Billington, C. K., & Hall, I. P. (2011). Real time analysis of β(2)-adrenoceptor-mediated signaling kinetics in human primary airway smooth muscle cells reveals both ligand and dose dependent differences. *Respiratory Research 12*, 89. https://doi.org/10.1186/1465-9921-12-89.

Billington, C. K., Le Jeune, I. R., Young, K. W., & Hall, I. P. (2008). A major functional role for phosphodiesterase 4D5 in human airway smooth muscle cells. *American Journal of Respiratory Cell and Molecular Biology 38*(1), 1–7. https://doi.org/10.1165/rcmb.2007-0171OC.

Billington, C. K., Ojo, O. O., Penn, R. B., & Ito, S. (2013). cAMP Regulation of Airway Smooth Muscle Function. *Pulmonary Pharmacology & Therapeutics 26*(1), 112–120. https://doi.org/10.1016/j.pupt.2012.05.007.

BinMahfouz, H., Borthakur, B., Yan, D., George, T., Giembycz, M. A., & Newton, R. (2015). Superiority of combined phosphodiesterase PDE3/PDE4 inhibition over PDE4 inhibition alone on glucocorticoid- and long-acting β2-adrenoceptor agonist-induced gene expression in human airway epithelial cells. *Molecular Pharmacology 87*(1), 64–76. https://doi.org/10.1124/mol.114.093393.

Blanchard, E., Zlock, L., Lao, A., Mika, D., Namkung, W., Xie, M., ... Richter, W. (2014). Anchored PDE4 regulates chloride conductance in wild-type and ΔF508-CFTR human airway epithelia. *The FASEB Journal 28*(2), 791–801. https://doi.org/10.1096/fj.13-240861.

Blanco, I., Santos, S., Gea, J., Güell, R., Torres, F., Gimeno-Santos, E., ... Barberà, J. A. (2013). Sildenafil to improve respiratory rehabilitation outcomes in COPD: a controlled trial. *The European Respiratory Journal 42*(4), 982–992. https://doi.org/10.1183/09031936.00176312.

Blease, K., Burke-Gaffney, A., & Hellewell, P. G. (1998). Modulation of cell adhesion molecule expression and function on human lung microvascular endothelial cells by inhibition of phosphodiesterases 3 and 4. *British Journal of Pharmacology 124*(1), 229–237. https://doi.org/10.1038/sj.bjp.0701833.

Bloom, T. J., & Beavo, J. A. (1996). Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. *Proceedings of the National Academy of Sciences of the United States of America 93*(24), 14188–14192.

Bolger, G., Michaeli, T., Martins, T., St John, T., Steiner, B., Rodgers, L., ... Ferguson, K. (1993). A family of human phosphodiesterases homologous to the dunce learning and memory gene product of Drosophila melanogaster are potential targets for antidepressant drugs. *Molecular and Cellular Biology 13*(10), 6558–6571.

Bolger, G. B., Erdogan, S., Jones, R. E., Loughney, K., Scotland, G., Hoffmann, R., ... Houslay, M. D. (1997). Characterization of five different proteins produced by alternatively spliced mRNAs from the human cAMP-specific phosphodiesterase PDE4D gene. *The Biochemical Journal 328*(Pt 2), 539–548.

Bolger, G. B., McPhee, I., & Houslay, M. D. (1996). Alternative splicing of cAMP-specific phosphodiesterase mRNA transcripts. Characterization of a novel tissue-specific isoform, RNPDE4A8. *The Journal of Biological Chemistry 271*(2), 1065–1071.

Boswell-Smith, V., Spina, D., Oxford, A. W., Comer, M. B., Seeds, E. A., & Page, C. P. (2006). The pharmacology of two novel long-acting phosphodiesterase 3/4 inhibitors, RPL554 [9,10-dimethoxy-2(2,4,6-trimethylphenylimino)-3-(n-carbamoyl-2-aminoethyl)-3,4,6,7-tetrahydro-2H-pyrimido[6,1-a]isoquinolin-4-one] and RPL565 [6,7-dihydro-2-(2,6-diisopropylphenoxy)-9,10-dimethoxy-4H-pyrimido[6,1-a]isoquinolin-4-one]. *The Journal of Pharmacology and Experimental Therapeutics 318*(2), 840–848. https://doi.org/10.1124/jpet.105.099192.

de Brito, J. M., Mauad, T., Cavalheiro, G. F., Yoshizaki, K., de André, P. A., Lichtenfels, A. J. F. C., ... Saldiva, P. H. N. (2018). Acute exposure to diesel and sewage biodiesel exhaust causes pulmonary and systemic inflammation in mice. *The Science of the Total Environment 628–629*, 1223–1233. https://doi.org/10.1016/j.scitotenv.2018.02.019.

Brown, D. M., Hutchison, L., Donaldson, K., MacKenzie, S. J., Dick, C. A. J., & Stone, V. (2007). The effect of oxidative stress on macrophages and lung epithelial cells: the role of phosphodiesterases 1 and 4. *Toxicology Letters 168*(1), 1–6. https://doi.org/10.1016/j.toxlet.2006.10.016.

Brunnée, T., Engelstätter, R., Steinijans, V. W., & Kunkel, G. (1992). Bronchodilatory effect of inhaled zardaverine, a phosphodiesterase III and IV inhibitor, in patients with asthma. *The European Respiratory Journal 5*(8), 982–985.

Bubb, K. J., Trinder, S. L., Baliga, R. S., Patel, J., Clapp, L. H., MacAllister, R. J., & Hobbs, A. J. (2014). Inhibition of phosphodiesterase 2 augments cGMP and cAMP signaling to ameliorate pulmonary hypertension. *Circulation 130*(6), 496–507. https://doi.org/10.1161/CIRCULATIONAHA.114.009751.

Buenestado, A., Grassin-Delyle, S., Guitard, F., Naline, E., Faisy, C., Israël-Biet, D., ... Devillier, P. (2012). Roflumilast inhibits the release of chemokines and TNF-α from human lung macrophages stimulated with lipopolysaccharide. *British Journal of Pharmacology 165*(6), 1877–1890. https://doi.org/10.1111/j.1476-5381.2011.01667.x.

Burgess, J. K., Oliver, B. G. G., Poniris, M. H., Ge, Q., Boustany, S., Cox, N., ... Black, J. L. (2006). A phosphodiesterase 4 inhibitor inhibits matrix protein deposition in airways in vitro. *The Journal of Allergy and Clinical Immunology 118*(3), 649–657. https://doi.org/10.1016/j.jaci.2006.05.019.

Calverley, P. M. A., Rabe, K. F., Goehring, U. -M., Kristiansen, S., Fabbri, L. M., Martinez, F. J., & M2-124 and M2-125 study groups (2009). Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials. *Lancet (London, England) 374*(9691), 685–694. https://doi.org/10.1016/S0140-6736(09)61255-1.

Calverley, P. M. A., Sanchez-Toril, F., McIvor, A., Teichmann, P., Bredenbroeker, D., & Fabbri, L. M. (2007). Effect of 1-year treatment with roflumilast in severe chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 176*(2), 154–161. https://doi.org/10.1164/rccm.200610-1563OC.

Calzetta, L., Cazzola, M., Page, C. P., Rogliani, P., Facciolo, F., & Matera, M. G. (2015). Pharmacological characterization of the interaction between the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554 and glycopyrronium on human isolated bronchi and small airways. *Pulmonary Pharmacology & Therapeutics 32*, 15–23. https://doi.org/10.1016/j.pupt.2015.03.007.

Calzetta, L., Page, C. P., Spina, D., Cazzola, M., Rogliani, P., Facciolo, F., & Matera, M. G. (2013). Effect of the mixed phosphodiesterase 3/4 inhibitor RPL554 on human isolated bronchial smooth muscle tone. *The Journal of Pharmacology and Experimental Therapeutics 346*(3), 414–423. https://doi.org/10.1124/jpet.113.204644.

Carman, C. V., & Martinelli, R. (2015). T Lymphocyte–Endothelial Interactions: Emerging Understanding of Trafficking and Antigen-Specific Immunity. *Frontiers in Immunology 6*. https://doi.org/10.3389/fimmu.2015.00603.

Castaño, T., Wang, H., Campillo, N. E., Ballester, S., González-García, C., Hernández, J., ... Gil, C. (2009). Synthesis, structural analysis, and biological evaluation of thioxoquinazoline derivatives as phosphodiesterase 7 inhibitors. *ChemMedChem 4*(5), 866–876. https://doi.org/10.1002/cmdc.200900043.

Castro, A., Abasolo, M. I., Gil, C., Segarra, V., & Martinez, A. (2001). CoMFA of benzyl derivatives of 2,1,3-benzo and benzothieno[3,2-alpha]thiadiazine 2,2-dioxides: clues for the design of phosphodiesterase 7 inhibitors. *European Journal of Medicinal Chemistry 36*(4), 333–338.

Chaouat, A., Naeije, R., & Weitzenblum, E. (2008). Pulmonary hypertension in COPD. *The European Respiratory Journal 32*(5), 1371–1385. https://doi.org/10.1183/09031936.00015608.

Cheung, Y. -F., Kan, Z., Garrett-Engele, P., Gall, I., Murdoch, H., Baillie, G. S., ... Castle, J. C. (2007). PDE4B5, a novel, super-short, brain-specific cAMP phosphodiesterase-4 variant whose isoform-specifying N-terminal region is identical to that of cAMP phosphodiesterase-4D6 (PDE4D6). *The Journal of Pharmacology and Experimental Therapeutics 322*(2), 600–609. https://doi.org/10.1124/jpet.107.122218.

Chevalier, E., Lagente, V., Dupont, M., Fargeau, H., Palazzi, X., Richard, V., ... Bertrand, C. P. (2012). Lack of involvement of type 7 phosphodiesterase in an experimental model of asthma. *The European Respiratory Journal 39*(3), 582–588. https://doi.org/10.1183/09031936.00102610.

Chung, K. (2006). Phosphodiesterase inhibitors in airways disease. *European Journal of Pharmacology 533*(1–3), 110–117. https://doi.org/10.1016/j.ejphar.2005.12.059.

Cong, M., Perry, S. J., Lin, F. T., Fraser, I. D., Hu, L. A., Chen, W., ... Lefkowitz, R. J. (2001). Regulation of membrane targeting of the G protein-coupled receptor kinase 2 by protein kinase A and its anchoring protein AKAP79. *The Journal of Biological Chemistry 276*(18), 15192–15199. https://doi.org/10.1074/jbc.M009130200.

Conti, M., & Beavo, J. (2007). Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annual Review of Biochemistry 76*, 481–511. https://doi.org/10.1146/annurev.biochem.76.060305.150444.

De Grove, K. C., Provoost, S., Braun, H., Blomme, E. E., Teufelberger, A. R., Krysko, O., ... Maes, T. (2018). IL-33 signalling contributes to pollutant-induced allergic airway inflammation. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology*. https://doi.org/10.1111/cea.13261.

Degerman, E., Belfrage, P., & Manganiello, V. C. (1997). Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *The Journal of Biological Chemistry 272*(11), 6823–6826.

Dekkers, B. G. J., Racké, K., & Schmidt, M. (2013). Distinct PKA and Epac compartmentalization in airway function and plasticity. *Pharmacology & Therapeutics 137*(2), 248–265. https://doi.org/10.1016/j.pharmthera.2012.10.006.

Dent, G., White, S. R., Tenor, H., Bodtke, K., Schudt, C., Leff, A. R., ... Rabe, K. F. (1998). Cyclic nucleotide phosphodiesterase in human bronchial epithelial cells: characterization of isoenzymes and functional effects of PDE inhibitors. *Pulmonary Pharmacology & Therapeutics 11*(1), 47–56.

Dong, H., Osmanova, V., Epstein, P. M., & Brocke, S. (2006). Phosphodiesterase 8 (PDE8) regulates chemotaxis of activated lymphocytes. *Biochemical and Biophysical Research Communications 345*(2), 713–719. https://doi.org/10.1016/j.bbrc.2006.04.143.

Dunkern, T. R., Feurstein, D., Rossi, G. A., Sabatini, F., & Hatzelmann, A. (2007). Inhibition of TGF-beta induced lung fibroblast to myofibroblast conversion by phosphodiesterase inhibiting drugs and activators of soluble guanylyl cyclase. *European Journal of Pharmacology 572*(1), 12–22. https://doi.org/10.1016/j.ejphar.2007.06.036.

Edwards, M. R., Facchinetti, F., Civelli, M., Villetti, G., & Johnston, S. L. (2016). Anti-inflammatory effects of the novel inhaled phosphodiesterase type 4 inhibitor CHF6001 on virus-inducible cytokines. *Pharmacology Research & Perspectives 4*(1), e00202. https://doi.org/10.1002/prp2.202.

Engels, P., Fichtel, K., & Lübbert, H. (1994). Expression and regulation of human and rat phosphodiesterase type IV isogenes. *FEBS Letters 350*(2–3), 291–295.

Engels, P., Sullivan, M., Müller, T., & Lübbert, H. (1995). Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). *FEBS Letters 358*(3), 305–310.

Fabbri, L. M., Calverley, P. M. A., Izquierdo-Alonso, J. L., Bundschuh, D. S., Brose, M., Martinez, F. J., ... M2-127 and M2-128 study groups (2009). Roflumilast in moderate-to-severe chronic obstructive pulmonary disease treated with longacting bronchodilators: two randomised clinical trials. *Lancet (London, England) 374*(9691), 695–703. https://doi.org/10.1016/S0140-6736(09)61252-6.

Factor, P., Akhmedov, A. T., McDonald, J. D., Qu, A., Wu, J., Jiang, H., ... Miller, R. L. (2011). Polycyclic aromatic hydrocarbons impair function of β2-adrenergic receptors in airway epithelial and smooth muscle cells. *American Journal of Respiratory Cell and Molecular Biology 45*(5), 1045–1049. https://doi.org/10.1165/rcmb.2010-0499OC.

Fisher, D. A., Smith, J. F., Pillar, J. S., St Denis, S. H., & Cheng, J. B. (1998). Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochemical and Biophysical Research Communications 246*(3), 570–577. https://doi.org/10.1006/bbrc.1998.8684.

Fortin, M., D'Anjou, H., Higgins, M. -E., Gougeon, J., Aubé, P., Moktefi, K., ... Ferrari, N. (2009). A multi-target antisense approach against PDE4 and PDE7 reduces smoke-induced lung inflammation in mice. *Respiratory Research 10*, 39. https://doi.org/10.1186/1465-9921-10-39.

Foster, R. W., Rakshi, K., Carpenter, J. R., & Small, R. C. (1992). Trials of the bronchodilator activity of the isoenzyme-selective phosphodiesterase inhibitor AH 21-132 in healthy volunteers during a methacholine challenge test. *British Journal of Clinical Pharmacology 34*(6), 527–534.

Franciosi, L. G., Diamant, Z., Banner, K. H., Zuiker, R., Morelli, N., Kamerling, I. M. C., ... Page, C. P. (2013). Efficacy and safety of RPL554, a dual PDE3 and PDE4 inhibitor, in healthy volunteers and in patients with asthma or chronic obstructive pulmonary disease: findings from four clinical trials. *The Lancet. Respiratory Medicine 1*(9), 714–727. https://doi.org/10.1016/S2213-2600(13)70187-5.

Gamanuma, M., Yuasa, K., Sasaki, T., Sakurai, N., Kotera, J., & Omori, K. (2003). Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. *Cellular Signalling 15*(6), 565–574.

Gantner, F., Götz, C., Gekeler, V., Schudt, C., Wendel, A., & Hatzelmann, A. (1998). Phosphodiesterase profile of human B lymphocytes from normal and atopic donors and the effects of PDE inhibition on B cell proliferation. *British Journal of Pharmacology 123*(6), 1031–1038. https://doi.org/10.1038/sj.bjp.0701688.

Gantner, F., Schudt, C., Wendel, A., & Hatzelmann, A. (1999). Characterization of the phosphodiesterase (PDE) pattern of in vitro-generated human dendritic cells (DC) and the influence of PDE inhibitors on DC function. *Pulmonary Pharmacology & Therapeutics 12*(6), 377–386. https://doi.org/10.1006/pupt.1999.0220.

Gardner, C., Robas, N., Cawkill, D., & Fidock, M. (2000). Cloning and characterization of the human and mouse PDE7B, a novel cAMP-specific cyclic nucleotide phosphodiesterase. *Biochemical and Biophysical Research Communications 272*(1), 186–192. https://doi.org/10.1006/bbrc.2000.2743.

Gauvreau, G. M., Boulet, L. -P., Schmid-Wirlitsch, C., Côté, J., Duong, M., Killian, K. J., ... O'Byrne, P. M. (2011). Roflumilast attenuates allergen-induced inflammation in mild asthmatic subjects. *Respiratory Research 12*, 140. https://doi.org/10.1186/1465-9921-12-140.

Ghofrani, H. A., Osterloh, I. H., & Grimminger, F. (2006). Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nature Reviews. Drug Discovery 5*(8), 689–702. https://doi.org/10.1038/nrd2030.

Giembycz, M. A. (2005). Life after PDE4: overcoming adverse events with dual-specificity phosphodiesterase inhibitors. *Current Opinion in Pharmacology 5*(3), 238–244. https://doi.org/10.1016/j.coph.2005.04.001.

Giembycz, M. A., Corrigan, C. J., Seybold, J., Newton, R., & Barnes, P. J. (1996). Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human CD4+ and CD8+ T-lymphocytes: role in regulating proliferation and the biosynthesis of interleukin-2. *British Journal of Pharmacology 118*(8), 1945–1958.

Giembycz, M. A., & Maurice, D. H. (2014). Cyclic nucleotide-based therapeutics for chronic obstructive pulmonary disease. *Current Opinion in Pharmacology 16*, 89–107. https://doi.org/10.1016/j.coph.2014.04.001.

Giembycz, M. A., & Smith, S. J. (2006). Phosphodiesterase 7A: a new therapeutic target for alleviating chronic inflammation? *Current Pharmaceutical Design 12*(25), 3207–3220.

Giembycz, M. A., & Newton, R. (2011). Harnessing the clinical efficacy of phosphodiesterase 4 inhibitors in inflammatory lung diseases: dual-selective phosphodiesterase inhibitors and novel combination therapies. *Handbook of Experimental Pharmacology 204*, 415–446. https://doi.org/10.1007/978-3-642-17969-3_18.

Glavas, N. A., Ostenson, C., Schaefer, J. B., Vasta, V., & Beavo, J. A. (2001). T cell activation up-regulates cyclic nucleotide phosphodiesterases 8A1 and 7A3. *Proceedings of the National Academy of Sciences of the United States of America 98*(11), 6319–6324. https://doi.org/10.1073/pnas.101131098.

Grootendorst, D. C., Gauw, S. A., Verhoosel, R. M., Sterk, P. J., Hospers, J. J., Bredenbröker, D., ... Rabe, K. F. (2007). Reduction in sputum neutrophil and eosinophil numbers by the PDE4 inhibitor roflumilast in patients with COPD. *Thorax 62*(12), 1081–1087. https://doi.org/10.1136/thx.2006.075937.

Guerra, S. (2009). Asthma and chronic obstructive pulmonary disease. *Current Opinion in Allergy and Clinical Immunology 9*(5), 409–416. https://doi.org/10.1097/ACI.0b013e3283300baf.

Haddad, J. J., Land, S. C., Tarnow-Mordi, W. O., Zembala, M., Kowalczyk, D., & Lauterbach, R. (2002). Immunopharmacological potential of selective phosphodiesterase inhibition. I. Differential regulation of lipopolysaccharide-mediated proinflammatory cytokine (interleukin-6 and tumor necrosis factor-alpha) biosynthesis in alveolar epithelial cells. *The Journal of Pharmacology and Experimental Therapeutics 300*(2), 559–566.

Hart, J. E., Eisen, E. A., & Laden, F. (2012). Occupational diesel exhaust exposure as a risk factor for chronic obstructive pulmonary disease. *Current Opinion in Pulmonary Medicine 18*(2), 151–154. https://doi.org/10.1097/MCP.0b013e32834f0eaa.

Hart, J. E., Laden, F., Schenker, M. B., & Garshick, E. (2006). Chronic obstructive pulmonary disease mortality in diesel-exposed railroad workers. *Environmental Health Perspectives 114*(7), 1013–1017. https://doi.org/10.1289/ehp.8743.

Hatzelmann, A., & Schudt, C. (2001). Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. *The Journal of Pharmacology and Experimental Therapeutics 297*(1), 267–279.

Havekes, R., Park, A. J., Tolentino, R. E., Bruinenberg, V. M., Tudor, J. C., Lee, Y., ... Abel, T. (2016). Compartmentalized PDE4A5 signaling impairs hippocampal synaptic plasticity and long-term memory. *The Journal of Neuroscience 36*(34), 8936–8946. https://doi.org/10.1523/JNEUROSCI.0248-16.2016.

Hayashi, M., Matsushima, K., Ohashi, H., Tsunoda, H., Murase, S., Kawarada, Y., & Tanaka, T. (1998). Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isozyme of 3′,5′-cyclic nucleotide phosphodiesterase. *Biochemical and Biophysical Research Communications 250*(3), 751–756. https://doi.org/10.1006/bbrc.1998.9379.

Hogg, J. C., & Timens, W. (2009). The pathology of chronic obstructive pulmonary disease. *Annual Review of Pathology 4*, 435–459. https://doi.org/10.1146/annurev.pathol.4.110807.092145.

Holguin, F. (2013). Oxidative stress in airway diseases. *Annals of the American Thoracic Society 10*(Suppl), S150–S157. https://doi.org/10.1513/AnnalsATS.201305-116AW.

Huston, E., Lumb, S., Russell, A., Catterall, C., Ross, A. H., Steele, M. R., ... Houslay, M. D. (1997). Molecular cloning and transient expression in COS7 cells of a novel human PDE4B cAMP-specific phosphodiesterase, HSPDE4B3. *The Biochemical Journal 328*(Pt 2), 549–558.

Hwang, T. -L., Tang, M. -C., Kuo, L. -M., Chang, W. -D., Chung, P. -J., Chang, Y. -W., & Fang, Y. -C. (2012). YC-1 potentiates cAMP-induced CREB activation and nitric oxide production in alveolar macrophages. *Toxicology and Applied Pharmacology 260*(2), 193–200. https://doi.org/10.1016/j.taap.2012.02.011.

Jin, S. -L. C., Goya, S., Nakae, S., Wang, D., Bruss, M., Hou, C., ... Conti, M. (2010). Phosphodiesterase 4B is essential for T(H)2-cell function and development of airway hyperresponsiveness in allergic asthma. *The Journal of Allergy and Clinical Immunology 126*(6). https://doi.org/10.1016/j.jaci.2010.08.014 1252-1259.e12.

Johnston, L. A., Erdogan, S., Cheung, Y. F., Sullivan, M., Barber, R., Lynch, M. J., ... Houslay, M. D. (2004). Expression, intracellular distribution and basis for lack of catalytic activity of the PDE4A7 isoform encoded by the human PDE4A cAMP-specific phosphodiesterase gene. *The Biochemical Journal 380*(Pt 2), 371–384. https://doi.org/10.1042/BJ20031662.

Johnstone, T. B., Smith, K. H., Koziol-White, C. J., Li, F., Kazarian, A. G., Corpuz, M. L., ... Ostrom, R. S. (2018). PDE8 is expressed in human airway smooth muscle and selectively regulates cAMP signaling by β2-adrenergic receptors and adenylyl cyclase 6. *American Journal of Respiratory Cell and Molecular Biology 58*(4), 530–541. https://doi.org/10.1165/rcmb.2017-0294OC.

Jones, N. A., Boswell-Smith, V., Lever, R., & Page, C. P. (2005). The effect of selective phosphodiesterase isoenzyme inhibition on neutrophil function in vitro. *Pulmonary Pharmacology & Therapeutics 18*(2), 93–101. https://doi.org/10.1016/j.pupt.2004.10.001.

Jones, N. A., Leport, M., Holand, T., Vos, T., Morgan, M., Fink, M., ... Page, C. P. (2007). Phosphodiesterase (PDE) 7 in inflammatory cells from patients with asthma and COPD. *Pulmonary Pharmacology & Therapeutics 20*(1), 60–68. https://doi.org/10.1016/j.pupt.2005.11.010.

Kadoshima-Yamaoka, K., Murakawa, M., Goto, M., Tanaka, Y., Inoue, H., Murafuji, H., ... Nishimura, C. (2009). Effect of phosphodiesterase 7 inhibitor ASB16165 on development and function of cytotoxic T lymphocyte. *International Immunopharmacology 9*(1), 97–102. https://doi.org/10.1016/j.intimp.2008.10.005.

Kaneko, T., Alvarez, R., Ueki, I. F., & Nadel, J. A. (1995). Elevated intracellular cyclic AMP inhibits chemotaxis in human eosinophils. *Cellular Signalling 7*(5), 527–534.

Kim, S. W., Kim, J. H., Park, C. K., Kim, T. J., Lee, S. Y., Kim, Y. K., ... Yoon, H. K. (2016). Effect of roflumilast on airway remodelling in a murine model of chronic asthma. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 46*(5), 754–763. https://doi.org/10.1111/cea.12670.

Kirkham, P., & Rahman, I. (2006). Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacology & Therapeutics 111*(2), 476–494. https://doi.org/10.1016/j.pharmthera.2005.10.015.

Kogiso, H., Hosogi, S., Ikeuchi, Y., Tanaka, S., Shimamoto, C., Matsumura, H., ... Nakahari, T. (2017). A low [Ca2+]i-induced enhancement of cAMP-activated ciliary beating by PDE1A inhibition in mouse airway cilia. *Pflügers Archiv / European Journal of Physiology 469*(9), 1215–1227. https://doi.org/10.1007/s00424-017-1988-9.

Kohyama, T., Liu, X., Wen, F. -Q., Zhu, Y. K., Wang, H., Kim, H. J., ... Rennard, S. I. (2002). PDE4 inhibitors attenuate fibroblast chemotaxis and contraction of native collagen gels. *American Journal of Respiratory Cell and Molecular Biology 26*(6), 694–701. https://doi.org/10.1165/ajrcmb.26.6.4743.

Kohyama, T., Liu, X., Zhu, Y. K., Wen, F. -Q., Wang, H. J., Fang, Q., ... Rennard, S. I. (2002). Phosphodiesterase 4 inhibitor cilomilast inhibits fibroblast-mediated collagen gel degradation induced by tumor necrosis factor-alpha and neutrophil elastase. *American Journal of Respiratory Cell and Molecular Biology 27*(4), 487–494. https://doi.org/10.1165/rcmb.4818.

Kolosionek, E., Savai, R., Ghofrani, H. A., Weissmann, N., Guenther, A., Grimminger, F., ... Pullamsetti, S. S. (2009). Expression and Activity of Phosphodiesterase Isoforms during Epithelial Mesenchymal Transition: The Role of Phosphodiesterase 4. *Molecular Biology of the Cell 20*(22), 4751–4765. https://doi.org/10.1091/mbc.E09-01-0019.

Konrad, F. M., Bury, A., Schick, M. A., Ngamsri, K. -C., & Reutershan, J. (2015). The unrecognized effects of phosphodiesterase 4 on epithelial cells in pulmonary inflammation. *PLoS One 10*(4), e0121725. https://doi.org/10.1371/journal.pone.0121725.

Kotera, J., Fujishige, K., Imai, Y., Kawai, E., Michibata, H., Akatsuka, H., ... Omori, K. (1999). Genomic origin and transcriptional regulation of two variants of cGMP-binding cGMP-specific phosphodiesterases. *European Journal of Biochemistry 262*(3), 866–873.

Kubo, S., Kobayashi, M., Iwata, M., Miyata, K., Takahashi, K., & Shimizu, Y. (2012). Antineutrophilic inflammatory activity of ASP3258, a novel phosphodiesterase type 4 inhibitor. *International Immunopharmacology 12*(1), 59–63. https://doi.org/10.1016/j.intimp.2011.10.011.

Kubo, S., Kobayashi, M., Iwata, M., Miyata, K., Takahashi, K., Miyata, K., & Shimizu, Y. (2011). Disease-modifying effect of ASP3258, a novel phosphodiesterase type 4 inhibitor, on subchronic cigarette smoke exposure-induced lung injury in guinea pigs. *European Journal of Pharmacology 659*(1), 79–84. https://doi.org/10.1016/j.ejphar.2011.02.042.

Lagente, V., Pruniaux, M. P., Junien, J. L., & Moodley, I. (1995). Modulation of cytokine-induced eosinophil infiltration by phosphodiesterase inhibitors. *American Journal of Respiratory and Critical Care Medicine 151*(6), 1720–1724. https://doi.org/10.1164/ajrccm.151.6.7539326.

Lambert, J. A., Raju, S. V., Tang, L. P., McNicholas, C. M., Li, Y., Courville, C. A., ... Rowe, S. M. (2014). Cystic fibrosis transmembrane conductance regulator activation by

roflumilast contributes to therapeutic benefit in chronic bronchitis. *American Journal of Respiratory Cell and Molecular Biology 50*(3), 549–558. https://doi.org/10.1165/rcmb.2013-0228OC.

Lea, S., Metryka, A., Facchinetti, F., & Singh, D. (2011). Increased expression of phosphodiesterase 4 (PDE4) A, B and D in alveolar macrophages from chronic obstructive pulmonary disease (COPD) patients. *European Respiratory Journal 38*(Suppl. 55), 217.

Leclerc, O., Lagente, V., Planquois, J. -M., Berthelier, C., Artola, M., Eichholtz, T., ... Schmidlin, F. (2006). Involvement of MMP-12 and phosphodiesterase type 4 in cigarette smoke-induced inflammation in mice. *The European Respiratory Journal 27*(6), 1102–1109. https://doi.org/10.1183/09031936.06.00076905.

Lee, R., Wolda, S., Moon, E., Esselstyn, J., Hertel, C., & Lerner, A. (2002). PDE7A is expressed in human B-lymphocytes and is up-regulated by elevation of intracellular cAMP. *Cellular Signalling 14*(3), 277–284.

Li, L., Yee, C., & Beavo, J. A. (1999). CD3- and CD28-dependent induction of PDE7 required for T cell activation. *Science (New York, N.Y.) 283*(5403), 848–851.

Liu, J., Muñoz, N. M., Meliton, A. Y., Zhu, X., Lambertino, A. T., Xu, C., ... Leff, A. R. (2004). Beta2-integrin adhesion caused by eotaxin but not IL-5 is blocked by PDE-4 inhibition and beta2-adrenoceptor activation in human eosinophils. *Pulmonary Pharmacology & Therapeutics 17*(2), 73–79. https://doi.org/10.1016/j.pupt.2003.10.005.

Mackenzie, K. F., Topping, E. C., Bugaj-Gaweda, B., Deng, C., Cheung, Y. -F., Olsen, A. E., ... Bolger, G. B. (2008). Human PDE4A8, a novel brain-expressed PDE4 cAMP-specific phosphodiesterase that has undergone rapid evolutionary change. *The Biochemical Journal 411*(2), 361–369. https://doi.org/10.1042/BJ20071251.

MacKenzie, S. J., Baillie, G. S., McPhee, I., Bolger, G. B., & Houslay, M. D. (2000). ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH2-terminal UCR regions. *The Journal of Biological Chemistry 275*(22), 16609–16617.

MacKenzie, S. J., Baillie, G. S., McPhee, I., MacKenzie, C., Seamons, R., McSorley, T., ... Houslay, M. D. (2002). Long PDE4 cAMP specific phosphodiesterases are activated by protein kinase A-mediated phosphorylation of a single serine residue in Upstream Conserved Region 1 (UCR1). *British Journal of Pharmacology 136*(3), 421–433. https://doi.org/10.1038/sj.bjp.0704743.

Manning, C. D., Burman, M., Christensen, S. B., Cieslinski, L. B., Essayan, D. M., Grous, M., ... Barnette, M. S. (1999). Suppression of human inflammatory cell function by subtype-selective PDE4 inhibitors correlates with inhibition of PDE4A and PDE4B. *British Journal of Pharmacology 128*(7), 1393–1398. https://doi.org/10.1038/sj.bjp.0702911.

Mariotti, F., Govoni, M., Lucci, G., Santoro, D., & Nandeuil, M. A. (2018). Safety, tolerability, and pharmacokinetics of single and repeat ascending doses of CHF6001, a novel inhaled phosphodiesterase-4 inhibitor: two randomized trials in healthy volunteers. *International Journal of Chronic Obstructive Pulmonary Disease 13*, 3399–3410. https://doi.org/10.2147/COPD.S174156.

Martín-Álvarez, R., Paúl-Fernández, N., Palomo, V., Gil, C., Martínez, A., & Mengod, G. (2017). A preliminary investigation of phoshodiesterase 7 inhibitor VP3.15 as therapeutic agent for the treatment of experimental autoimmune encephalomyelitis mice. *Journal of Chemical Neuroanatomy 80*, 27–36. https://doi.org/10.1016/j.jchemneu.2016.12.001.

Martínez, A., Castro, A., Gil, C., Miralpeix, M., Segarra, V., Doménech, T., ... Puigdoménech, P. (2000). Benzyl derivatives of 2,1,3-benzo- and benzothieno[3,2-a]thiadiazine 2,2-dioxides: first phosphodiesterase 7 inhibitors. *Journal of Medicinal Chemistry 43*(4), 683–689.

Martinez, F. J., Calverley, P. M. A., Goehring, U. -M., Brose, M., Fabbri, L. M., & Rabe, K. F. (2015). Effect of roflumilast on exacerbations in patients with severe chronic obstructive pulmonary disease uncontrolled by combination therapy (REACT): a multicentre randomised controlled trial. *Lancet (London, England) 385*(9971), 857–866. https://doi.org/10.1016/S0140-6736(14)62410-7.

Martinez, F. J., Rabe, K. F., Sethi, S., Pizzichini, E., McIvor, A., Anzueto, A., ... Rennard, S. I. (2016). Effect of Roflumilast and Inhaled Corticosteroid/Long-Acting β2-Agonist on Chronic Obstructive Pulmonary Disease Exacerbations (RE(2)SPOND). A Randomized Clinical Trial. *American Journal of Respiratory and Critical Care Medicine 194*(5), 559–567. https://doi.org/10.1164/rccm.201607-1349OC.

Martinez, S. E., Wu, A. Y., Glavas, N. A., Tang, X. -B., Turley, S., Hol, W. G. J., & Beavo, J. A. (2002). The two GAF domains in phosphodiesterase 2A have distinct roles in dimerization and in cGMP binding. *Proceedings of the National Academy of Sciences of the United States of America 99*(20), 13260–13265. https://doi.org/10.1073/pnas.192374899.

Martorana, P. A., Beume, R., Lucattelli, M., Wollin, L., & Lungarella, G. (2005). Roflumilast fully prevents emphysema in mice chronically exposed to cigarette smoke. *American Journal of Respiratory and Critical Care Medicine 172*(7), 848–853. https://doi.org/10.1164/rccm.200411-1549OC.

Mauad, T., & Dolhnikoff, M. (2008). Pathologic similarities and differences between asthma and chronic obstructive pulmonary disease. *Current Opinion in Pulmonary Medicine 14*(1), 31–38. https://doi.org/10.1097/MCP.0b013e3282f19846.

McCluskey, K., Klein, U., Linnevers, C., Ji, Y. -H., Yang, A., Husfeld, C., & Thomas, G. R. (2006). Phosphodiesterase type 4 inhibitors cause proinflammatory effects in vivo. *The Journal of Pharmacology and Experimental Therapeutics 319*(1), 468–476. https://doi.org/10.1124/jpet.106.105080.

McLaughlin, M. M., Cieslinski, L. B., Burman, M., Torphy, T. J., & Livi, G. P. (1993). A low-Km, rolipram-sensitive, cAMP-specific phosphodiesterase from human brain. Cloning and expression of cDNA, biochemical characterization of recombinant protein, and tissue distribution of mRNA. *The Journal of Biological Chemistry 268*(9), 6470–6476.

McPhee, I., Pooley, L., Lobban, M., Bolger, G., & Houslay, M. D. (1995). Identification, characterization and regional distribution in brain of RPDE-6 (RNPDE4A5), a novel splice variant of the PDE4A cyclic AMP phosphodiesterase family. *Biochemical Journal 310*(Pt 3), 965–974.

de Medeiros, A. S., Wyman, A. R., Alaamery, M. A., Allain, C., Ivey, F. D., Wang, L., ... Hoffman, C. S. (2017). Identification and characterization of a potent and biologically-active PDE4/7 inhibitor via fission yeast-based assays. *Cellular Signalling* 40, 73–80. https://doi.org/10.1016/j.cellsig.2017.08.011.

Medvedova, I., Prso, M., Eichlerova, A., Mokra, D., Mikolka, P., & Mokry, J. (2015). Influence of roflumilast on airway reactivity and apoptosis in ovalbumin-sensitized Guinea pigs. *Advances in Experimental Medicine and Biology* 838, 11–18. https://doi.org/10.1007/5584_2014_77.

Méhats, C., Jin, S. -L C., Wahlstrom, J., Law, E., Umetsu, D. T., & Conti, M. (2003). PDE4D plays a critical role in the control of airway smooth muscle contraction. *The FASEB Journal* 17(13), 1831–1841. https://doi.org/10.1096/fj.03-0274com.

Merrill, R. A., & Strack, S. (2014). Mitochondria: a kinase anchoring protein 1, a signaling platform for mitochondrial form and function. *The International Journal of Biochemistry & Cell Biology* 48, 92–96. https://doi.org/10.1016/j.biocel.2013.12.012.

Mestre, L., Redondo, M., Carrillo-Salinas, F. J., Morales-García, J. A., Alonso-Gil, S., Pérez-Castillo, A., ... Guaza, C. (2015). PDE7 inhibitor TC3.6 ameliorates symptomatology in a model of primary progressive multiple sclerosis. *British Journal of Pharmacology* 172(17), 4277–4290. https://doi.org/10.1111/bph.13192.

Meurs, H., Gosens, R., & Zaagsma, J. (2008). Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models. *The European Respiratory Journal* 32(2), 487–502. https://doi.org/10.1183/09031936.00023608.

Milara, J., Armengot, M., Bañuls, P., Tenor, H., Beume, R., Artigues, E., & Cortijo, J. (2012). Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. *British Journal of Pharmacology* 166(8), 2243–2262. https://doi.org/10.1111/j.1476-5381.2012.01929.x.

Milara, J., Lluch, J., Almudever, P., Freire, J., Xiaozhong, Q., & Cortijo, J. (2014). Roflumilast N-oxide reverses corticosteroid resistance in neutrophils from patients with chronic obstructive pulmonary disease. *The Journal of Allergy and Clinical Immunology* 134(2), 314–322. https://doi.org/10.1016/j.jaci.2014.02.001.

Milara, J., Navarro, A., Almudéver, P., Lluch, J., Morcillo, E. J., & Cortijo, J. (2011). Oxidative stress-induced glucocorticoid resistance is prevented by dual PDE3/PDE4 inhibition in human alveolar macrophages. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 41(4), 535–546. https://doi.org/10.1111/j.1365-2222.2011.03715.x.

Milara, J., Peiró, T., Serrano, A., Artigues, E., Aparicio, J., Tenor, H., ... Cortijo, J. (2015). Simvastatin increases the ability of roflumilast N-oxide to inhibit cigarette smoke-induced epithelial to mesenchymal transition in well-differentiated human bronchial epithelial cells in vitro. *COPD* 12(3), 320–331. https://doi.org/10.3109/15412555.2014.948995.

Milara, J., Peiró, T., Serrano, A., Guijarro, R., Zaragozá, C., Tenor, H., & Cortijo, J. (2014). Roflumilast N-oxide inhibits bronchial epithelial to mesenchymal transition induced by cigarette smoke in smokers with COPD. *Pulmonary Pharmacology & Therapeutics* 28(2), 138–148. https://doi.org/10.1016/j.pupt.2014.02.001.

Millen, J., MacLean, M. R., & Houslay, M. D. (2006). Hypoxia-induced remodelling of PDE4 isoform expression and cAMP handling in human pulmonary artery smooth muscle cells. *European Journal of Cell Biology* 85(7), 679–691. https://doi.org/10.1016/j.ejcb.2006.01.006.

Miró, X., Casacuberta, J. M., Gutiérrez-López, M. D., de Landázuri, M. O., & Puigdomènech, P. (2000). Phosphodiesterases 4D and 7A splice variants in the response of HUVEC cells to TNF-alpha(1). *Biochemical and Biophysical Research Communications* 274(2), 415–421. https://doi.org/10.1006/bbrc.2000.3146.

Mokra, D., Drgova, A., Pullmann, R., & Calkovska, A. (2012). Selective phosphodiesterase 3 inhibitor olprinone attenuates meconium-induced oxidative lung injury. *Pulmonary Pharmacology & Therapeutics* 25(3), 216–222. https://doi.org/10.1016/j.pupt.2012.02.007.

Mokry, J., Joskova, M., Mokra, D., Christensen, I., & Nosalova, G. (2013). Effects of selective inhibition of PDE4 and PDE7 on airway reactivity and cough in healthy and ovalbumin-sensitized guinea pigs. *Advances in Experimental Medicine and Biology* 756, 57–64. https://doi.org/10.1007/978-94-007-4549-0_8.

Mokrý, J., Urbanová, A., Medvedová, I., Kertys, M., Mikolka, P., Kosutová, P., & Mokrá, D. (2016). Effects of selective inhibition of PDE4 by YM976 on airway reactivity and cough in ovalbumin-sensitized Guinea pigs. *Advances in Experimental Medicine and Biology* 921, 61–70. https://doi.org/10.1007/5584_2016_237.

Mokry, J., Urbanova, A., Medvedova, I., Kertys, M., Mikolka, P., Kosutova, P., & Mokra, D. (2017). Effects of tadalafil (PDE5 inhibitor) and roflumilast (PDE4 inhibitor) on airway reactivity and markers of inflammation in ovalbumin-induced airway hyperresponsiveness in guinea pigs. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society* 68(5), 721–730.

Momose, T., Okubo, Y., Horie, S., Suzuki, J., Isobe, M., & Sekiguchi, M. (1998). Effects of intracellular cyclic AMP modulators on human eosinophil survival, degranulation and CD11b expression. *International Archives of Allergy and Immunology* 117(2), 138–145. https://doi.org/10.1159/000024001.

Morales-Garcia, J. A., Redondo, M., Alonso-Gil, S., Gil, C., Perez, C., Martinez, A., ... Perez-Castillo, A. (2011). Phosphodiesterase 7 inhibition preserves dopaminergic neurons in cellular and rodent models of Parkinson disease. *PLoS One* 6(2), e17240. https://doi.org/10.1371/journal.pone.0017240.

Mori, H., Nose, T., Ishitani, K., Kasagi, S., Souma, S., Akiyoshi, T., ... Seyama, K. (2008). Phosphodiesterase 4 inhibitor GPD-1116 markedly attenuates the development of cigarette smoke-induced emphysema in senescence-accelerated mice P1 strain. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 294(2), L196–L204. https://doi.org/10.1152/ajplung.00173.2007.

Movsesian, M., Ahmad, F., & Hirsch, E. (2018). Functions of PDE3 Isoforms in Cardiac Muscle. *Journal of Cardiovascular Development and Disease* 5(1). https://doi.org/10.3390/jcdd5010010.

Murray, F., Patel, H. H., Suda, R. Y. S., Zhang, S., Thistlethwaite, P. A., Yuan, J. X. -J., & Insel, P. A. (2007). Expression and activity of cAMP phosphodiesterase isoforms in pulmonary artery smooth muscle cells from patients with pulmonary hypertension: role for PDE1. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 292(1), L294–L303. https://doi.org/10.1152/ajplung.00190.2006.

Musheshe, N., Schmidt, M., & Zaccolo, M. (2018). cAMP: from long-range second messenger to nanodomain signalling. *Trends in Pharmacological Sciences* 39(2), 209–222. https://doi.org/10.1016/j.tips.2017.11.006.

Nakata, A., Ogawa, K., Sasaki, T., Koyama, N., Wada, K., Kotera, J., ... Kaminuma, O. (2002). Potential role of phosphodiesterase 7 in human T cell function: comparative effects of two phosphodiesterase inhibitors. *Clinical and Experimental Immunology* 128(3), 460–466.

Nejman-Gryz, P., Grubek-Jaworska, H., Glapiński, J., Hoser, G., & Chazan, R. (2006). Effects of the phosphodiestrase-4 inhibitor rolipram on lung resistance and inflammatory reaction in experimental asthma. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society* 57(Suppl. 4), 229–239.

Nicholson, C. D., Shahid, M., Bruin, J., Barron, E., Spiers, I., de Boer, J., ... Dent, G. (1995). Characterization of ORG 20241, a combined phosphodiesterase IV/III cyclic nucleotide phosphodiesterase inhibitor for asthma. *The Journal of Pharmacology and Experimental Therapeutics* 274(2), 678–687.

Nikolaev, V. O., Bünemann, M., Hein, L., Hannawacker, A., & Lohse, M. J. (2004). Novel single chain cAMP sensors for receptor-induced signal propagation. *The Journal of Biological Chemistry* 279(36), 37215–37218. https://doi.org/10.1074/jbc.C400302200.

Nikolova, S., Guenther, A., Savai, R., Weissmann, N., Ghofrani, H. A., Konigshoff, M., ... Pullamsetti, S. S. (2010). Phosphodiesterase 6 subunits are expressed and altered in idiopathic pulmonary fibrosis. *Respiratory Research* 11, 146. https://doi.org/10.1186/1465-9921-11-146.

Obernolte, R., Bhakta, S., Alvarez, R., Bach, C., Zuppan, P., Mulkins, M., ... Shelton, E. R. (1993). The cDNA of a human lymphocyte cyclic-AMP phosphodiesterase (PDE IV) reveals a multigene family. *Gene* 129(2), 239–247.

Obernolte, R., Ratzliff, J., Baecker, P. A., Daniels, D. V., Zuppan, P., Jarnagin, K., & Shelton, E. R. (1997). Multiple splice variants of phosphodiesterase PDE4C cloned from human lung and testis. *Biochimica et Biophysica Acta* 1353(3), 287–297.

Oldenburger, A., Maarsingh, H., & Schmidt, M. (2012). Multiple facets of cAMP signalling and physiological impact: cAMP compartmentalization in the lung. *Pharmaceuticals* 5(12), 1291–1331. https://doi.org/10.3390/ph5121291.

Omori, K., & Kotera, J. (2007). Overview of PDEs and their regulation. *Circulation Research* 100(3), 309–327. https://doi.org/10.1161/01.RES.0000256354.95791.f1.

Otero, C., Peñaloza, J. P., Rodas, P. I., Fernández-Ramires, R., Velasquez, L., & Jung, J. E. (2014). Temporal and spatial regulation of cAMP signaling in disease: role of cyclic nucleotide phosphodiesterases. *Fundamental & Clinical Pharmacology* 28(6), 593–607. https://doi.org/10.1111/fcp.12080.

Owens, R. J., Lumb, S., Rees-Milton, K., Russell, A., Baldock, D., Lang, V., ... Perry, M. J. (1997). Molecular cloning and expression of a human phosphodiesterase 4C. *Cellular Signalling* 9(8), 575–585.

Page, C. P. (2014). Phosphodiesterase inhibitors for the treatment of asthma and chronic obstructive pulmonary disease. *International Archives of Allergy and Immunology* 165(3), 152–164. https://doi.org/10.1159/000368800.

Page, C. P., & Spina, D. (2012). Selective PDE inhibitors as novel treatments for respiratory diseases. *Current Opinion in Pharmacology* 12(3), 275–286. https://doi.org/10.1016/j.coph.2012.02.016.

Park, J. -W., Ryter, S. W., Kyung, S. Y., Lee, S. P., & Jeong, S. H. (2013). The phosphodiesterase 4 inhibitor rolipram protects against cigarette smoke extract-induced apoptosis in human lung fibroblasts. *European Journal of Pharmacology* 706(1–3), 76–83. https://doi.org/10.1016/j.ejphar.2013.02.049.

Patel, N. S., Klett, J., Pilarzyk, K., Lee, D. I., Kass, D., Menniti, F. S., & Kelly, M. P. (2018). Identification of new PDE9A isoforms and how their expression and subcellular compartmentalization in the brain change across the life span. *Neurobiology of Aging* 65, 217–234. https://doi.org/10.1016/j.neurobiolaging.2018.01.019.

Paterniti, I., Mazzon, E., Gil, C., Impellizzeri, D., Palomo, V., Redondo, M., ... Cuzzocrea, S. (2011). PDE 7 inhibitors: new potential drugs for the therapy of spinal cord injury. *PLoS One* 6(1), e15937. https://doi.org/10.1371/journal.pone.0015937.

Patrucco, E., Albergine, M. S., Santana, L. F., & Beavo, J. A. (2010). Phosphodiesterase 8A (PDE8A) regulates excitation-contraction coupling in ventricular myocytes. *Journal of Molecular and Cellular Cardiology* 49(2), 330–333. https://doi.org/10.1016/j.yjmcc.2010.03.016.

Pavlaki, N., & Nikolaev, V. O. (2018). Imaging of PDE2- and PDE3-Mediated cGMP-to-cAMP Cross-Talk in Cardiomyocytes. *Journal of Cardiovascular Development and Disease* 5(1). https://doi.org/10.3390/jcdd5010004.

PDE2 inhibition: Potential for the treatment of cognitive disorders (2013). *Bioorganic & Medicinal Chemistry Letters* 23(24), 6522–6527. https://doi.org/10.1016/j.bmcl.2013.10.014.

Perez-Gonzalez, R., Pascual, C., Antequera, D., Bolos, M., Redondo, M., Perez, D. I., ... Carro, E. (2013). Phosphodiesterase 7 inhibitor reduced cognitive impairment and pathological hallmarks in a mouse model of Alzheimer's disease. *Neurobiology of Aging* 34(9), 2133–2145. https://doi.org/10.1016/j.neurobiolaging.2013.03.011.

Pérez-Torres, S., Cortés, R., Tolnay, M., Probst, A., Palacios, J. M., & Mengod, G. (2003). Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. *Experimental Neurology* 182(2), 322–334.

Perez-Zoghbi, J. F., Bai, Y., & Sanderson, M. J. (2010). Nitric oxide induces airway smooth muscle cell relaxation by decreasing the frequency of agonist-induced Ca2+ oscillations. *The Journal of General Physiology* 135(3), 247–259. https://doi.org/10.1085/jgp.200910365.

Peter, D., Jin, S. L. C., Conti, M., Hatzelmann, A., & Zitt, C. (2007). Differential expression and function of phosphodiesterase 4 (PDE4) subtypes in human primary CD4+ T

cells: predominant role of PDE4D. *Journal of Immunology (Baltimore, Md. : 1950) 178* (8), 4820–4831.

Pfeifer, A., Kilić, A., & Hoffmann, L. S. (2013). Regulation of metabolism by cGMP. *Pharmacology & Therapeutics 140*(1), 81–91. https://doi.org/10.1016/j.pharmthera.2013.06.001.

Profita, M., Chiappara, G., Mirabella, F., Di Giorgi, R., Chimenti, L., Costanzo, G., ... Vignola, A. M. (2003). Effect of cilomilast (Ariflo) on TNF-alpha, IL-8, and GM-CSF release by airway cells of patients with COPD. *Thorax 58*(7), 573–579.

Rabe, K. F., Tenor, H., Dent, G., Schudt, C., Liebig, S., & Magnussen, H. (1993). Phosphodiesterase isozymes modulating inherent tone in human airways: identification and characterization. *The American Journal of Physiology 264*(5 Pt 1), L458–L464. https://doi.org/10.1152/ajplung.1993.264.5.L458.

Rabe, K. F., Bateman, E. D., O'Donnell, D., Witte, S., Bredenbröker, D., & Bethke, T. D. (2005). Roflumilast–an oral anti-inflammatory treatment for chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet (London, England) 366*(9485), 563–571. https://doi.org/10.1016/S0140-6736(05)67100-0.

Raju, S. V., Rasmussen, L., Sloane, P. A., Tang, L. P., Libby, E. F., & Rowe, S. M. (2017). Roflumilast reverses CFTR-mediated ion transport dysfunction in cigarette smoke-exposed mice. *Respiratory Research*, 18. https://doi.org/10.1186/s12931-017-0656-0.

Reddel, H. K., Bateman, E. D., Becker, A., Boulet, L. -P., Cruz, A. A., Drazen, J. M., ... FitzGerald, J. M. (2015). A summary of the new GINA strategy: a roadmap to asthma control. *The European Respiratory Journal 46*(3), 622–639. https://doi.org/10.1183/13993003.00853-2015.

Reinhardt, R. R., Chin, E., Zhou, J., Taira, M., Murata, T., Manganiello, V. C., & Bondy, C. A. (1995). Distinctive anatomical patterns of gene expression for cGMP-inhibited cyclic nucleotide phosphodiesterases. *The Journal of Clinical Investigation 95*(4), 1528–1538. https://doi.org/10.1172/JCI117825.

Rena, G., Begg, F., Ross, A., MacKenzie, C., McPhee, I., Campbell, L., ... Houslay, M. D. (2001). Molecular cloning, genomic positioning, promoter identification, and characterization of the novel cyclic amp-specific phosphodiesterase PDE4A10. *Molecular Pharmacology 59*(5), 996–1011.

Ricciardolo, F. L. M., Sterk, P. J., Gaston, B., & Folkerts, G. (2004). Nitric oxide in health and disease of the respiratory system. *Physiological Reviews 84*(3), 731–765. https://doi.org/10.1152/physrev.00034.2003.

Richter, W., & Conti, M. (2002). Dimerization of the type 4 cAMP-specific phosphodiesterases is mediated by the upstream conserved regions (UCRs). *The Journal of Biological Chemistry 277*(43), 40212–40221. https://doi.org/10.1074/jbc.M203585200.

Richter, W., Jin, S. -L. C., & Conti, M. (2005). Splice variants of the cyclic nucleotide phosphodiesterase PDE4D are differentially expressed and regulated in rat tissue. *The Biochemical Journal 388*(Pt 3), 803–811. https://doi.org/10.1042/BJ20050030.

Rieder, F., Siegmund, B., Bundschuh, D. S., Lehr, H. -A., Endres, S., & Eigler, A. (2013). The selective phosphodiesterase 4 inhibitor roflumilast and phosphodiesterase 3/4 inhibitor pumafentrine reduce clinical score and TNF expression in experimental colitis in mice. *PLoS One 8*(2), e56867. https://doi.org/10.1371/journal.pone.0056867.

Rudd, R. M., Gellert, A. R., Studdy, P. R., & Geddes, D. M. (1983). Inhibition of exercise-induced asthma by an orally absorbed mast cell stabilizer (M & B 22,948). *British Journal of Diseases of the Chest 77*(1), 78–86.

Sachs, B. D., Baillie, G. S., McCall, J. R., Passino, M. A., Schachtrup, C., Wallace, D. A., ... Akassoglou, K. (2007). p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. *The Journal of Cell Biology 177*(6), 1119–1132. https://doi.org/10.1083/jcb.200701040.

Safavi, M., Baeeri, M., & Abdollahi, M. (2013). New methods for the discovery and synthesis of PDE7 inhibitors as new drugs for neurological and inflammatory disorders. *Expert Opinion on Drug Discovery 8*(6), 733–751. https://doi.org/10.1517/17460441.2013.787986.

Said, S. I., Hamidi, S. A., & Gonzalez Bosc, L. (2010). Asthma and pulmonary arterial hypertension: do they share a key mechanism of pathogenesis? *The European Respiratory Journal 35*(4), 730–734. https://doi.org/10.1183/09031936.00097109.

Sasaki, T., Kotera, J., Yuasa, K., & Omori, K. (2000). Identification of human PDE7B, a cAMP-specific phosphodiesterase. *Biochemical and Biophysical Research Communications 271* (3), 575–583. https://doi.org/10.1006/bbrc.2000.2661.

Sayner, S. L. (2011). Emerging themes of cAMP regulation of the pulmonary endothelial barrier. *American Journal of Physiology - Lung Cellular and Molecular Physiology 300* (5), L667–L678. https://doi.org/10.1152/ajplung.00433.2010.

Schermuly, R. T., Pullamsetti, S. S., Kwapiszewska, G., Dumitrascu, R., Tian, X., Weissmann, N., ... Grimminger, F. (2007). Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy. *Circulation 115*(17), 2331–2339. https://doi.org/10.1161/CIRCULATIONAHA.106.676809.

Schindler, R. F. R., & Brand, T. (2016). The Popeye domain containing protein family – A novel class of cAMP effectors with important functions in multiple tissues. *Progress in Biophysics and Molecular Biology 120*(1–3), 28–36. https://doi.org/10.1016/j.pbiomolbio.2016.01.001.

Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J. S., Campos, M., Krick, S., & Salathe, M. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. *Respiratory Research 16*, 135. https://doi.org/10.1186/s12931-015-0294-3.

Schmidt, C. J., Chapin, D. S., Cianfrogna, J., Corman, M. L., Hajos, M., Harms, J. F., ... Menniti, F. S. (2008). Preclinical Characterization of Selective Phosphodiesterase 10A Inhibitors: A New Therapeutic Approach to the Treatment of Schizophrenia. *Journal of Pharmacology and Experimental Therapeutics 325*(2), 681–690. https://doi.org/10.1124/jpet.107.132910.

Schmidt, D. T., Watson, N., Dent, G., Rühlmann, E., Branscheid, D., Magnussen, H., & Rabe, K. F. (2000). The effect of selective and non-selective phosphodiesterase inhibitors on allergen- and leukotriene C(4)-induced contractions in passively sensitized human airways. *British Journal of Pharmacology 131*(8), 1607–1618. https://doi.org/10.1038/sj.bjp.0703725.

Sebkhi, A., Strange, J. W., Phillips, S. C., Wharton, J., & Wilkins, M. R. (2003). Phosphodiesterase type 5 as a target for the treatment of hypoxia-induced pulmonary hypertension. *Circulation 107*(25), 3230–3235. https://doi.org/10.1161/01.CIR.0000074226.20466.B1.

Seehase, S., Lauenstein, H. -D., Schlumbohm, C., Switalla, S., Neuhaus, V., Förster, C., ... Knauf, S. (2012). LPS-induced lung inflammation in marmoset monkeys - an acute model for anti-inflammatory drug testing. *PLoS One 7*(8), e43709. https://doi.org/10.1371/journal.pone.0043709.

Seimetz, M., Parajuli, N., Pichl, A., Bednorz, M., Ghofrani, H. A., Schermuly, R. T., ... Weissmann, N. (2015). Cigarette Smoke-Induced Emphysema and Pulmonary Hypertension Can Be Prevented by Phosphodiesterase 4 and 5 Inhibition in Mice. *PLoS One 10*(6), e0129327. https://doi.org/10.1371/journal.pone.0129327.

Seldon, P. M., Barnes, P. J., Meja, K., & Giembycz, M. A. (1995). Suppression of lipopolysaccharide-induced tumor necrosis factor-alpha generation from human peripheral blood monocytes by inhibitors of phosphodiesterase 4: interaction with stimulants of adenylyl cyclase. *Molecular Pharmacology 48*(4), 747–757.

Seldon, P. M., & Giembycz, M. A. (2001). Suppression of granulocyte/macrophage colony-stimulating factor release from human monocytes by cyclic AMP-elevating drugs: role of interleukin-10. *British Journal of Pharmacology 134*(1), 58–67. https://doi.org/10.1038/sj.bjp.0704238.

Selige, J., Hatzelmann, A., & Dunkern, T. (2011). The differential impact of PDE4 subtypes in human lung fibroblasts on cytokine-induced proliferation and myofibroblast conversion. *Journal of Cellular Physiology 226*(8), 1970–1980. https://doi.org/10.1002/jcp.22529.

Selige, J., Tenor, H., Hatzelmann, A., & Dunkern, T. (2010). Cytokine-dependent balance of mitogenic effects in primary human lung fibroblasts related to cyclic AMP signaling and phosphodiesterase 4 inhibition. *Journal of Cellular Physiology 223*(2), 317–326. https://doi.org/10.1002/jcp.22037.

Sette, C., & Conti, M. (1996). Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. *The Journal of Biological Chemistry 271*(28), 16526–16534.

Shahid, M., van Amsterdam, R. G., de Boer, J., ten Berge, R. E., Nicholson, C. D., & Zaagsma, J. (1991). The presence of five cyclic nucleotide phosphodiesterase isoenzyme activities in bovine tracheal smooth muscle and the functional effects of selective inhibitors. *British Journal of Pharmacology 104*(2), 471–477.

Shakur, Y., Holst, L. S., Landstrom, T. R., Movsesian, M., Degerman, E., & Manganiello, V. (2001). Regulation and function of the cyclic nucleotide phosphodiesterase (PDE3) gene family. *Progress in Nucleic Acid Research and Molecular Biology 66*, 241–277.

Shakur, Y., Wilson, M., Pooley, L., Lobban, M., Griffiths, S. L., Campbell, A. M., ... Houslay, M. D. (1995). Identification and characterization of the type-IVA cyclic AMP-specific phosphodiesterase RD1 as a membrane-bound protein expressed in cerebellum. *The Biochemical Journal 306*(Pt 3), 801–809.

Shepherd, M., McSorley, T., Olsen, A. E., Johnston, L. A., Thomson, N. C., Baillie, G. S., ... Bolger, G. B. (2003). Molecular cloning and subcellular distribution of the novel PDE4B4 cAMP-specific phosphodiesterase isoform. *Biochemical Journal 370*(Pt 2), 429–438. https://doi.org/10.1042/BJ20021082.

Sheppard, C. L., Lee, L. C. Y., Hill, E. V., Henderson, D. J. P., Anthony, D. F., Houslay, D. M., ... Houslay, M. D. (2014). Mitotic activation of the DISC1-inducible cyclic AMP phosphodiesterase-4D9 (PDE4D9), through multi-site phosphorylation, influences cell cycle progression. *Cellular Signalling 26*(9), 1958–1974. https://doi.org/10.1016/j.cellsig.2014.04.023.

Shimizu-Albergine, M., Tsai, L. -C. L., Patrucco, E., & Beavo, J. A. (2012). cAMP-specific phosphodiesterases 8A and 8B, essential regulators of Leydig cell steroidogenesis. *Molecular Pharmacology 81*(4), 556–566. https://doi.org/10.1124/mol.111.076125.

Silva, D., & Jacinto, T. (2016). Inhaled β2-agonists in asthma management: an evolving story. *Breathe (Sheffield, England) 12*(4), 375–377. https://doi.org/10.1183/20734735.017116.

Silva, P. M. R., Alves, A. C., Serra, M. F., Pires, A. L. A., Silva, J. P., Barreto, E. O., ... Martins, M. A. (2001). Modulation of eotaxin formation and eosinophil migration by selective inhibitors of phosphodiesterase type 4 isoenzyme. *British Journal of Pharmacology 134* (2), 283–294. https://doi.org/10.1038/sj.bjp.0704233.

Singh, D., Leaker, B., Boyce, M., Nandeuil, M. A., Collarini, S., Mariotti, F., ... Barnes, P. J. (2016). A novel inhaled phosphodiesterase 4 inhibitor (CHF6001) reduces the allergen challenge response in asthmatic patients. *Pulmonary Pharmacology & Therapeutics 40*, 1–6. https://doi.org/10.1016/j.pupt.2016.06.011.

Singh, S. P., Barrett, E. G., Kalra, R., Razani-Boroujerdi, S., Langley, R. J., Kurup, V., ... Sopori, M. L. (2003). Prenatal cigarette smoke decreases lung cAMP and increases airway hyperresponsiveness. *American Journal of Respiratory and Critical Care Medicine 168* (3), 342–347. https://doi.org/10.1164/rccm.200211-1262OC.

Singh, S. P., Mishra, N. C., Rir-sima-ah, J., Campen, M., Kurup, V., Razani-Boroujerdi, S., & Sopori, M. L. (2009). Maternal exposure to secondhand cigarette smoke primes the lung for induction of phosphodiesterase4D5 isozyme and exacerbated Th2 responses; rolipram attenuates the airway hyperreactivity and muscarinic receptor expression but not lung inflammation and atopy. *Journal of Immunology (Baltimore, Md. : 1950) 183*(3), 2115–2121. https://doi.org/10.4049/jimmunol.0900826.

Sisson, T. H., Christensen, P. J., Muraki, Y., Dils, A. J., Chibucos, L., Subbotina, N., ... Hazama, M. (2018). Phosphodiesterase 4 inhibition reduces lung fibrosis following targeted type II alveolar epithelial cell injury. *Physiological Reports 6*(12), e13753https://doi.org/10.14814/phy2.13753.

Smith, S. J., Brookes-Fazakerley, S., Donnelly, L. E., Barnes, P. J., Barnette, M. S., & Giembycz, M. A. (2003). Ubiquitous expression of phosphodiesterase 7A in human proinflammatory and immune cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology 284*(2), L279–L289. https://doi.org/10.1152/ajplung.00170.2002.

Smith, S. J., Cieslinski, L. B., Newton, R., Donnelly, L. E., Fenwick, P. S., Nicholson, A. G., ... Giembycz, M. A. (2004). Discovery of BRL 50481 [3-(N,N-dimethylsulfonamido)-4-

methyl-nitrobenzene], a selective inhibitor of phosphodiesterase 7: in vitro studies in human monocytes, lung macrophages, and CD8+ T-lymphocytes. *Molecular Pharmacology 66*(6), 1679–1689. https://doi.org/10.1124/mol.104.002246.

Snyder, P. B., Esselstyn, J. M., Loughney, K., Wolda, S. L., & Florio, V. A. (2005). The role of cyclic nucleotide phosphodiesterases in the regulation of adipocyte lipolysis. *Journal of Lipid Research 46*(3), 494–503. https://doi.org/10.1194/jlr.M400362-JLR200.

Soderling, S. H., Bayuga, S. J., & Beavo, J. A. (1998). Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. *Proceedings of the National Academy of Sciences of the United States of America 95*(15), 8991–8996.

Soderling, S. H., & Beavo, J. A. (2000). Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Current Opinion in Cell Biology 12*(2), 174–179.

Sohal, S. S., Reid, D., Soltani, A., Ward, C., Weston, S., Muller, H. K., ... Walters, E. H. (2010). Reticular basement membrane fragmentation and potential epithelial mesenchymal transition is exaggerated in the airways of smokers with chronic obstructive pulmonary disease. *Respirology 15*(6), 930–938. https://doi.org/10.1111/j.1440-1843.2010.01808.x.

Sohal, S. S., & Walters, E. H. (2013). Role of epithelial mesenchymal transition (EMT) in chronic obstructive pulmonary disease (COPD). *Respiratory Research 14*, 120. https://doi.org/10.1186/1465-9921-14-120.

Soltani, A., Reid, D. W., Sohal, S. S., Wood-Baker, R., Weston, S., Muller, H. K., & Walters, E. H. (2010). Basement membrane and vascular remodelling in smokers and chronic obstructive pulmonary disease: a cross-sectional study. *Respiratory Research 11*, 105. https://doi.org/10.1186/1465-9921-11-105.

Sousa, C. T., Brito, T. S., Lima, F. J. B., Siqueira, R. J. B., Magalhães, P. J. C., Lima, A. a. M., ... Havt, A. (2011). Sildenafil decreases rat tracheal hyperresponsiveness to carbachol and changes canonical transient receptor potential gene expression after antigen challenge. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas 44*(6), 562–572.

Southworth, T., Kaur, M., Hodgson, L., Facchinetti, F., Villetti, G., Civelli, M., & Singh, D. (2018). Anti-inflammatory effects of the phosphodiesterase type 4 inhibitor CHF6001 on bronchoalveolar lavage lymphocytes from asthma patients. *Cytokine*. https://doi.org/10.1016/j.cyto.2018.06.007.

Sprenger, J. U., & Nikolaev, V. O. (2013). Biophysical techniques for detection of cAMP and cGMP in living cells. *International Journal of Molecular Sciences 14*(4), 8025–8046. https://doi.org/10.3390/ijms14048025.

Sprenger, J. U., Perera, R. K., Steinbrecher, J. H., Lehnart, S. E., Maier, L. S., Hasenfuss, G., & Nikolaev, V. O. (2015). In vivo model with targeted cAMP biosensor reveals changes in receptor-microdomain communication in cardiac disease. *Nature Communications 6*, 6965. https://doi.org/10.1038/ncomms7965.

Steiner, S., Bisig, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2016). Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms. *Archives of Toxicology 90*(7), 1541–1553. https://doi.org/10.1007/s00204-016-1736-5.

Strahm, E., Rane, A., & Ekström, L. (2014). PDE7B is involved in nandrolone decanoate hydrolysis in liver cytosol and its transcription is up-regulated by androgens in HepG2. *Frontiers in Pharmacology 5*, 132. https://doi.org/10.3389/fphar.2014.00132.

Sullivan, M., Rena, G., Begg, F., Gordon, L., Olsen, A. S., & Houslay, M. D. (1998). Identification and characterization of the human homologue of the short PDE4A cAMP-specific phosphodiesterase RD1 (PDE4A1) by analysis of the human HSPDE4A gene locus located at chromosome 19p13.2. *The Biochemical Journal 333*(Pt 3), 693–703.

Sun, J. -G., Deng, Y. -M., Wu, X., Tang, H. -F., Deng, J. -F., Chen, J. -Q., ... Xie, Q. -M. (2006). Inhibition of phosphodiesterase activity, airway inflammation and hyperresponsiveness by PDE4 inhibitor and glucocorticoid in a murine model of allergic asthma. *Life Sciences 79*(22), 2077–2085. https://doi.org/10.1016/j.lfs.2006.07.001.

Tajima, T., Shinoda, T., Urakawa, N., Shimizu, K., & Kaneda, T. (2018). Phosphodiesterase 9 (PDE9) regulates bovine tracheal smooth muscle relaxation. *The Journal of Veterinary Medical Science 80*(3), 499–502. https://doi.org/10.1292/jvms.18-0011.

Tang, H. -F., Lu, J. -J., Tang, J. -F., Zheng, X., Liang, Y. -Q., Wang, X. -F., ... Chen, J. -Q. (2010). Action of a Novel PDE4 inhibitor ZL-n-91 on lipopolysaccharide-induced acute lung injury. *International Immunopharmacology 10*(4), 406–411. https://doi.org/10.1016/j.intimp.2010.01.003.

Tannheimer, S. L., Wright, C. D., & Salmon, M. (2012). Combination of roflumilast with a beta-2 adrenergic receptor agonist inhibits proinflammatory and profibrotic mediator release from human lung fibroblasts. *Respiratory Research 13*, 28. https://doi.org/10.1186/1465-9921-13-28.

Tao, J., & Malbon, C. C. (2008). G-protein-coupled receptor-associated A-kinase anchoring proteins AKAP5 and AKAP12: differential signaling to MAPK and GPCR recycling. *Journal of Molecular Signaling 3*, 19. https://doi.org/10.1186/1750-2187-3-19.

Tian, X., Vroom, C., Ghofrani, H. A., Weissmann, N., Bieniek, E., Grimminger, F., ... Pullamsetti, S. S. (2011). Phosphodiesterase 10A upregulation contributes to pulmonary vascular remodeling. *PLoS One 6*(4), e18136. https://doi.org/10.1371/journal.pone.0018136.

Torphy, T. J., Undem, B. J., Cieslinski, L. B., Luttmann, M. A., Reeves, M. L., & Hay, D. W. (1993). Identification, characterization and functional role of phosphodiesterase isozymes in human airway smooth muscle. *The Journal of Pharmacology and Experimental Therapeutics 265*(3), 1213–1223.

Toward, T. J., Smith, N., & Broadley, K. J. (2004). Effect of phosphodiesterase-5 inhibitor, sildenafil (Viagra), in animal models of airways disease. *American Journal of Respiratory and Critical Care Medicine 169*(2), 227–234. https://doi.org/10.1164/rccm.200211-1372OC.

Trian, T., Burgess, J. K., Niimi, K., Moir, L. M., Ge, Q., Berger, P., ... Oliver, B. G. (2011). β2-Agonist induced cAMP Is decreased in asthmatic airway smooth muscle due to increased PDE4D. *PLoS One 6*(5). https://doi.org/10.1371/journal.pone.0020000.

Tsai, L. -C. L., Shimizu-Albergine, M., & Beavo, J. A. (2011). The high-affinity cAMP-specific phosphodiesterase 8B controls steroidogenesis in the mouse adrenal gland. *Molecular Pharmacology 79*(4), 639–648. https://doi.org/10.1124/mol.110.069104.

Turner, M. J., Matthes, E., Billet, A., Ferguson, A. J., Thomas, D. Y., Randell, S. H., ... Hanrahan, J. W. (2016). The dual phosphodiesterase 3 and 4 inhibitor RPL554 stimulates CFTR and ciliary beating in primary cultures of bronchial epithelia. *American Journal of Physiology. Lung Cellular and Molecular Physiology 310*(1), L59–L70. https://doi.org/10.1152/ajplung.00324.2015.

Tyrrell, J., Qian, X., Freire, J., & Tarran, R. (2015). Roflumilast combined with adenosine increases mucosal hydration in human airway epithelial cultures after cigarette smoke exposure. *American Journal of Physiology. Lung Cellular and Molecular Physiology 308*(10), L1068–L1077. https://doi.org/10.1152/ajplung.00395.2014.

Ukena, D., Rentz, K., Reiber, C., & Sybrecht, G. W. (1995). Effects of the mixed phosphodiesterase III/IV inhibitor, zardaverine, on airway function in patients with chronic airflow obstruction. *Respiratory Medicine 89*(6), 441–444.

Urbanova, A., Medvedova, I., Kertys, M., Mikolka, P., Kosutova, P., Mokra, D., & Mokrý, J. (2017). Dose dependent effects of tadalafil and roflumilast on ovalbumin-induced airway hyperresponsiveness in guinea pigs. *Experimental Lung Research 43*(9–10), 407–416. https://doi.org/10.1080/01902148.2017.1386735.

Van Ly, D., De Pedro, M., James, P., Morgan, L., Black, J. L., Burgess, J. K., & Oliver, B. G. G. (2013). Inhibition of phosphodiesterase 4 modulates cytokine induction from toll like receptor activated, but not rhinovirus infected, primary human airway smooth muscle. *Respiratory Research 14*, 127. https://doi.org/10.1186/1465-9921-14-127.

Vang, A. G., Ben-Sasson, S. Z., Dong, H., Kream, B., DeNinno, M. P., Claffey, M. M., ... Brocke, S. (2010). PDE8 regulates rapid Teff cell adhesion and proliferation independent of ICER. *PLoS One 5*(8), e12011. https://doi.org/10.1371/journal.pone.0012011.

Vecchio, D., Acquaviva, A., Arezzini, B., Tenor, H., Martorana, P. A., & Gardi, C. (2013). Downregulation of NOX4 expression by roflumilast N-oxide reduces markers of fibrosis in lung fibroblasts. *Mediators of Inflammation 2013*, 745984. https://doi.org/10.1155/2013/745984.

Venkatasamy, R., & Spina, D. (2016). Novel relaxant effects of RPL554 on guinea pig tracheal smooth muscle contractility. *British Journal of Pharmacology 173*(15), 2335–2351. https://doi.org/10.1111/bph.13512.

Vergne, F., Bernardelli, P., Lorthiois, E., Pham, N., Proust, E., Oliveira, C., ... Li, M. (2004). Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 2: metabolism-directed optimization studies towards orally bioavailable derivatives. *Bioorganic & Medicinal Chemistry Letters 14*(18), 4615–4621. https://doi.org/10.1016/j.bmcl.2004.07.009.

Villetti, G., Carnini, C., Battipaglia, L., Preynat, L., Bolzoni, P. T., Bassani, F., ... Civelli, M. (2015). CHF6001 II: a novel phosphodiesterase 4 inhibitor, suitable for topical pulmonary administration–in vivo preclinical pharmacology profile defines a potent anti-inflammatory compound with a wide therapeutic window. *The Journal of Pharmacology and Experimental Therapeutics 352*(3), 568–578. https://doi.org/10.1124/jpet.114.220558.

Violin, J. D., DiPilato, L. M., Yildirim, N., Elston, T. C., Zhang, J., & Lefkowitz, R. J. (2008). beta2-Adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real time cAMP dynamics. *The Journal of Biological Chemistry 283*(5), 2949–2961. https://doi.org/10.1074/jbc.M707009200.

Vogelmeier, C. F., Criner, G. J., Martinez, F. J., Anzueto, A., Barnes, P. J., Bourbeau, J., ... Agustí, A. (2017). Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD Executive Summary. *American Journal of Respiratory and Critical Care Medicine 195*(5), 557–582. https://doi.org/10.1164/rccm.201701-0218PP.

Wade, J. F., & Newman, L. S. (1993). Diesel asthma. Reactive airways disease following overexposure to locomotive exhaust. *Journal of Occupational Medicine: Official Publication of the Industrial Medical Association 35*(2), 149–154.

Wallace, D. A., Johnston, L. A., Huston, E., MacMaster, D., Houslay, T. M., Cheung, Y. -F., ... Houslay, M. D. (2005). Identification and characterization of PDE4A11, a novel, widely expressed long isoform encoded by the human PDE4A cAMP phosphodiesterase gene. *Molecular Pharmacology 67*(6), 1920–1934. https://doi.org/10.1124/mol.104.009423.

Wang, C., Xu, J., Yang, L., Xu, Y., Zhang, X., Bai, C., ... China Pulmonary Health Study Group (2018). Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross-sectional study. *Lancet (London, England) 391*(10131), 1706–1717. https://doi.org/10.1016/S0140-6736(18)30841-9.

Wang, D., Deng, C., Bugaj-Gaweda, B., Kwan, M., Gunwaldsen, C., Leonard, C., ... De Vivo, M. (2003). Cloning and characterization of novel PDE4D isoforms PDE4D6 and PDE4D7. *Cellular Signalling 15*(9), 883–891.

Wang, P., Wu, P., Egan, R. W., & Billah, M. M. (2000). Cloning, characterization, and tissue distribution of mouse phosphodiesterase 7A1. *Biochemical and Biophysical Research Communications 276*(3), 1271–1277. https://doi.org/10.1006/bbrc.2000.3613.

Watz, H., Mistry, S. J., Lazaar, A. L., & IPC101939 Investigators (2013). Safety and tolerability of the inhaled phosphodiesterase 4 inhibitor GSK256066 in moderate COPD. *Pulmonary Pharmacology & Therapeutics 26*(5), 588–595. https://doi.org/10.1016/j.pupt.2013.05.004.

Wedzicha, J. A. (2013). Dual PDE 3/4 inhibition: a novel approach to airway disease? *The Lancet. Respiratory Medicine 1*(9), 669–670. https://doi.org/10.1016/S2213-2600(13)70211-X.

Welte, T., & Groneberg, D. A. (2006). Asthma and COPD. *Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft Fur Toxikologische Pathologie 57*(Suppl. 2), 35–40. https://doi.org/10.1016/j.etp.2006.02.004.

Wilson, J. M., Ogden, A. M. L., Loomis, S., Gilmour, G., Baucum, A. J., Belecky-Adams, T. L., & Merchant, K. M. (2015). Phosphodiesterase 10A inhibitor, MP-10 (PF-2545920), produces greater induction of c-Fos in dopamine D2 neurons than in D1 neurons in the neostriatum. *Neuropharmacology 99*, 379–386. https://doi.org/10.1016/j.neuropharm.2015.08.008.

Witzenrath, M., Gutbier, B., Schmeck, B., Tenor, H., Seybold, J., Kuelzer, R., ... Schütte, H. (2009). Phosphodiesterase 2 inhibition diminished acute lung injury in murine

pneumococcal pneumonia. *Critical Care Medicine 37*(2), 584–590. https://doi.org/10.1097/CCM.0b013e3181959814.

Wright, L. C., Seybold, J., Robichaud, A., Adcock, I. M., & Barnes, P. J. (1998). Phosphodiesterase expression in human epithelial cells. *The American Journal of Physiology 275*(4 Pt 1), L694–L700.

Yan, Z., Wang, H., Cai, J., & Ke, H. (2009). Refolding and kinetic characterization of the phosphodiesterase-8A catalytic domain. *Protein Expression and Purification 64*(1), 82–88. https://doi.org/10.1016/j.pep.2008.10.005.

Yanaka, N., Kotera, J., Ohtsuka, A., Akatsuka, H., Imai, Y., Michibata, H., ... Omori, K. (1998). Expression, structure and chromosomal localization of the human cGMP-binding cGMP-specific phosphodiesterase PDE5A gene. *European Journal of Biochemistry 255* (2), 391–399.

Yang, G., McIntyre, K. W., Townsend, R. M., Shen, H. H., Pitts, W. J., Dodd, J. H., ... Watson, A. J. (2003). Phosphodiesterase 7A-deficient mice have functional T cells. *Journal of Immunology (Baltimore, Md. : 1950) 171*(12), 6414–6420.

Yoon, H. -K., Hu, H. -J., Rhee, C. -K., Shin, S. -H., Oh, Y. -M., Lee, S. -D., ... Chung, Y. -J. (2014). Polymorphisms in PDE4D are associated with a risk of COPD in non-emphysematous Koreans. *COPD 11*(6), 652–658. https://doi.org/10.3109/15412555.2014.898045.

Zhang, F., Zhang, L., Qi, Y., & Xu, H. (2016). Mitochondrial cAMP signaling. *Cellular and Molecular Life Sciences: CMLS 73*(24), 4577–4590. https://doi.org/10.1007/s00018-016-2282-2.

Zhang, X., Feng, Q., & Cote, R. H. (2005). Efficacy and selectivity of phosphodiesterase-targeted drugs to inhibit photoreceptor phosphodiesterase (PDE6) in retinal photoreceptors. *Investigative Ophthalmology & Visual Science 46*(9), 3060–3066. https://doi.org/10.1167/iovs.05-0257.

Zheng, J., Yang, J., Zhou, X., Zhao, L., Hui, F., Wang, H., ... Zhong, N. (2014). Roflumilast for the treatment of COPD in an Asian population: a randomized, double-blind, parallel-group study. *Chest 145*(1), 44–52. https://doi.org/10.1378/chest.13-1252.

Zhu, B., Lindsey, A., Li, N., Lee, K., Ramirez-Alcantara, V., Canzoneri, J. C., ... Piazza, G. A. (2017). Phosphodiesterase 10A is overexpressed in lung tumor cells and inhibitors selectively suppress growth by blocking β-catenin and MAPK signaling. *Oncotarget 8*(41), 69264–69280. https://doi.org/10.18632/oncotarget.20566.

Zuo, H., Han, B., Poppinga, W. J., Ringnalda, L., Kistemaker, L. E. M., Halayko, A. J., ... Schmidt, M. (2018). Cigarette smoke up-regulates PDE3 and PDE4 to decrease cAMP in airway cells. *British Journal of Pharmacology 175*(14), 2988–3006. https://doi.org/10.1111/bph.14347.