22

A Multi-Targeted Antisense Oligonucleotide-Based Therapy Directed at Phosphodiesterases 4 and 7 for COPD

Rosanne Seguin and Nicolay Ferrari
Topigen Pharmaceuticals Inc.,
Part of the Pharmaxis Ltd. Group, Montréal, QC
Canada

1. Introduction

Recent drug development for chronic obstructive pulmonary disease (COPD) has focused on strategies aimed at reducing the underlying inflammation by selective inhibition of phosphodiesterases (PDE), specifically the PDE4 isoforms. The anti-inflammatory and bronchodilator activities of PDE4 inhibitors have been well documented (Giembycz &Field 2010), however their clinical development has been hampered by their low therapeutic ratio and dose-dependent systemic side effects. PXS TPI1100 is an inhaled drug candidate consisting of two modified antisense oligonucleotides (AON) directed at PDE isoforms 4B, 4D and 7A. PXS TPI1100 has been designed to reduce the recruitment and persistence of inflammatory cells in COPD through an unique mechanism of action and has the potential to be a novel, highly effective approach for this respiratory disease.

In this chapter, we will present the rationale for the design of PXS TPI1100 including a summary of the PDE families and the proposed role they play in regulating inflammation in the lung. Next we will present an overview of the discovery and selection process for the drug candidate, including a summary of the key results from pre-clinical pharmacology, both *in vitro* models as well as two *in vivo* models of neutrophilic inflammation: cigarette smoke mouse model and LPS challenge model. These results will be compared to the first-in-class PDE4 inhibitor, roflumilast (Daxas/Daliresp). We shall conclude with the expected development plan for PXS TPI1100 including the design of upcoming clinical study trials.

2. Chronic obstructive pulmonary disease

COPD is a respiratory disease of airway obstruction and lung damage and is sometimes called chronic bronchitis and/or emphysema. COPD kills millions of people each year and it is currently the fourth leading cause of death worldwide, with forecasts to be the third leading cause by 2020 (ref www.goldcopd.com). COPD, as defined by the Global Initiative for Chronic Lung Disease (GOLD) is *"a preventable and treatable disease with some extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is*

usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" (Gold 2009). Symptoms of COPD include chronic cough, excessive sputum production, wheeze, shortness of breath and chest tightness. The 4 stages of COPD, designated as Mild, Moderate, Severe and Very Severe, are defined according to lung function as assessed by spirometry, usually the post-bronchodilator ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC). The cellular and molecular mechanisms that contribute to COPD pathogenesis remain incompletely understood yet it is believed that COPD is caused by underlying inflammation characterized by increased presence of neutrophils, macrophages and CD8+ T cells (Gold 2009). Products of neutrophils induce mucus hypersecretion and are implicated both in the generation of mucus metaplasia in chronic bronchitis and the destruction of lung tissue in emphysema. Macrophages are also sources of proteinases and antiproteinases in the lung, oxidative stress and mucus hypersecretion (Ward 2010). Exacerbations play a large role in the disease progression of COPD, and exacerbations become more frequent and more severe as COPD progresses (Hurst et al. 2010).

2.1 Traditional management of COPD

Currently, the only intervention known to influence the loss of lung function is smoking cessation (Gold 2009). Besides treating symptoms and improving quality of life, the treatment focus includes prevention of future exacerbations, reduction of mortality and prevention of disease progression. Treatment for COPD falls into two categories: those medications which relieve symptoms of airflow limitations and those medications which control the underlying inflammation. As such, the current gold standard of treatment for COPD patients involves a step-up paradigm commencing with short-acting bronchodilators (either short-acting β2 agonists or antimuscarinic agents), then adding on long-acting bronchodilators again either long-acting β2 agonists (LABA) or long acting muscarinics, (LAMA) followed by inclusion of inhaled corticosteroids (ICS). Lastly, long term oxygen and possible surgical treatments are final treatment options. Typically, the most common treatment involves ICS/LABA class of drugs, but can also include methylxanthines (bronchodilator) and leukotriene antagonists (anti-inflammatory) (Hurst et al. 2010). The majority of novel treatments for COPD forecasted to launch prior to 2018, are in fact minimally differentiated from current options, with either being improved dosing or combining therapies such as combinations of LABA/LAMA.

Another dilemma is that although highly effective in asthma, ICS have provided little therapeutic benefit in COPD (Barnes 2006). In patients with severe COPD, histological analysis of their peripheral airways have shown an intense inflammatory response, despite treatment with high doses of ICS, suggesting steroid resistance (Hogg et al. 2004). Combinations of ICS and LABA have been shown to be more effective at reducing COPD exacerbations (Calverley et al. 2007) but have not been shown to statistically decrease mortality (Calverley et al. 2007) (Tashkin et al. 2008). ICS use has been associated with osteoporosis, glaucoma, cataracts and skin thinning (Giembycz &Field 2010) and increased risk of pneumonia in patients with COPD (Ernst et al. 2007). Even with the current and immediate future medications, there are clear unmet needs for more effective anti-inflammatories in COPD both for reducing progression of the disease and reducing mortality.

2.2 Phosphodiesterases as targets for COPD

PDE4 is a member of the PDE family of enzymes whose function is to selectively catalyze the hydrolysis of cycle adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) (Bender &Beavo 2006). Second messengers perform intracellular signaling and cAMP is a key member. The level of cAMP can be regulated by its rate of degradation which is controlled by PDEs (Figure 1). As such, the regulation of PDEs is sophisticated and complex. This family currently includes 11 members (PDE1 to PDE11) of which there are multiple isoforms or splice variants. Several different PDEs can be expressed in a single cell type, and the localization of these PDEs within a cell regulates the local concentration of cAMP or cGMP. Besides being regulated through differential genetic expression, PDEs can be biochemically regulated by phosphorylation, binding of Ca2+/calmodulin and various protein-protein interactions (Bender &Beavo 2006). The PDEs with higher affinity for cAMP than cGMP include PDE3, PDE4, PDE7, PDE8 and PDE11 (similar affinities). These multiple isoforms and their differential expression across cell types

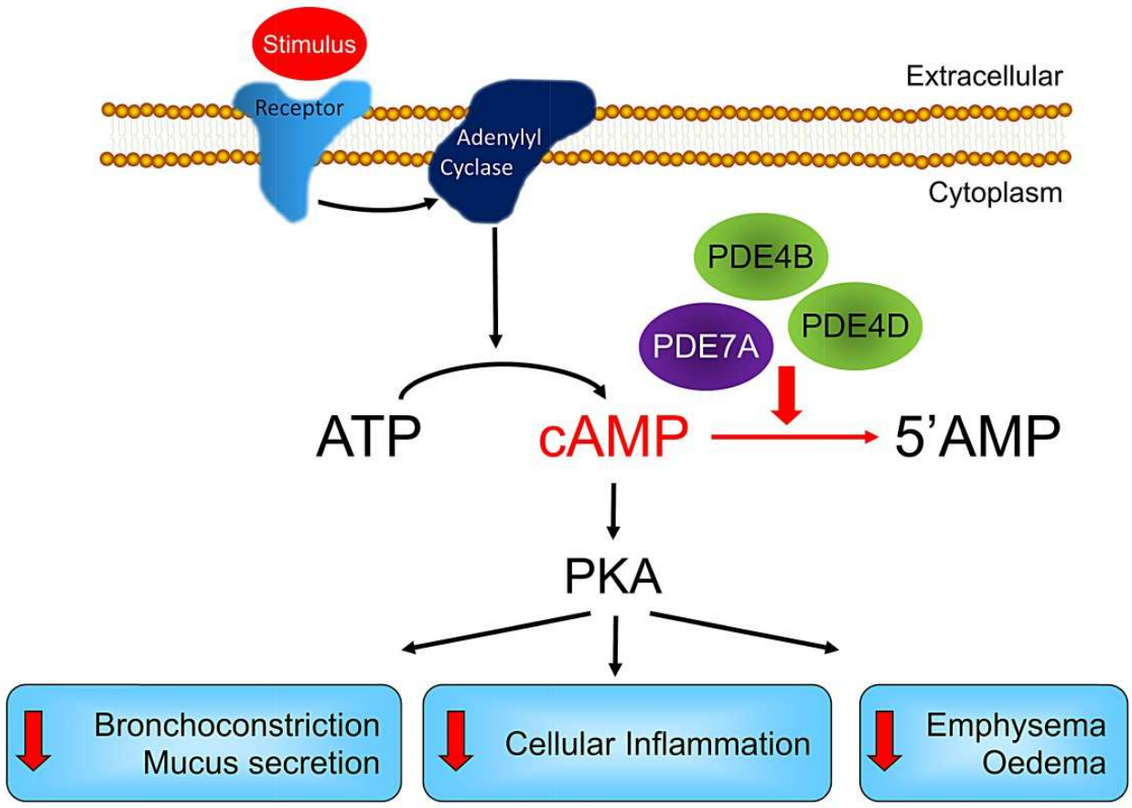

Fig. 1. Cartoon of the cAMP pathway, which is presumably activated upon binding of a stimuli to its receptor embedded in the cell membrane. Known components of this pathway include the calcium/calmodulin-activated adenylyl cyclase, the phosphodiesterase (PDE), and cAMP-dependent protein kinase (PKA) with its catalytic and regulatory subunits. Activation of PKA will lead to phosphorylation of cytoplasmic and nuclear targets. In the lung, inhibition of the PDE will lead to an elevation of the intracellular levels of cAMP resulting with a reduction of the bronchoconstriction, mucus secretion, cellular inflammation and in the long term decrease the emphysema/oedema.

are reasons PDEs are good drug targets as selective inhibition of a specific PDE isoform would limit nonspecific sides effects associated with broader PDE inhibition.

Another reason PDEs have been the focus of drug companies is based on the pharmacologic principle that a more rapid and larger percentage change in concentration is achieved through regulating the degradation of a second messenger than comparable regulation of the rates of synthesis (Bender &Beavo 2006). In most cells the levels of cAMP are between <1 to 10 μM which enables a competitive inhibitor to not need to compete with high levels of endogenous substrate to be effective, in contrast to many protein kinase inhibitors which need to have sufficient affinity to displace mM concentrations of ATP (Bender &Beavo 2006).

There are four PDE4 (A/B/C/D) genes which generate multiple variants as a result of splicing differences in their N termini (Bender &Beavo 2006). PDE4 isoforms, which are widely expressed in many tissues and cell types including the lung, have been shown to play a key role in macrophage and monocyte activation and functions, neutrophils infiltration and vasodilation (Table 1). There has been more information collated on PDE4 than other PDEs mostly from the work resulting from PDE4A, 4B and 4D knock out mice. In PDE4D knockout mice, their airways were shown to be refractory to cholinergic stimulation (Mehats et al. 2003) while PDE4B knockout mice were shown to have effects on immune cells (Jin &Conti 2002; Jin et al. 2005) and both genes were shown to be required for neutrophils recruitment in a model of lung injury in response to inhaled endotoxin (Ariga et al. 2004).

A new first-in class treatment, the PDE4 inhibitor Daxas/Daliresp (Nycomed), has recently been approved in Europe in 2010 and in the USA in 2011 for patients with severe COPD.

	Structural Lung Cells			Inflammatory Cells		
cAMP modulator	Lung Epithelium	Smooth Muscle	Epithelial Cells	Monocyte/ Macrophage	Neutrophils	T-Cells
PDE3	+	+	++	+++	+	+
PDE4	++	++	+++	++++	+++	++
PDE7	+++	+++	+++	+++	+	+
PXS TPI 1100	●	●	●	●	●	●

Table 1. Expression of different cAMP-modulating PDE isoforms in lung cells and inflammatory cells. PDE4 and PDE7 are highly expressed in lung structural cells as well as in inflammatory cells. Delivered to the lung, PXS TPI1100 can inhibit expression of PDE4 and PDE7 in both lung structural and inflammatory cells.

Daxas (3-cyclopropylmethoxy-4-difluoromethoxy-*N*-[3,5-di-chloropyrid-4-yl]-benzamide) is a once-a-day tablet, taken orally, whose principal action is to reduce inflammation. The clinical results from the six Phase III trials performed using Daxas will be reviewed below. Before, touting the benefits of PDE4 inhibitors in COPD, it is important to note that Daxas is not without its adverse events which include diarrhea, weight loss, nausea, headache and abdominal pain (Giembycz &Field 2010), which have been observed previously with other PDE4 inhibitor drugs (Down et al. 2006).

Like the PDE4 family, the PDE7 family, which consists of PDE7A and PDE7B, is highly selective for cAMP as a substrate (Bender &Beavo 2006). While the function of PDE7 has not been fully elucidated, PDE7 isoforms have been implicated in the activation of inflammatory cells (Li L et al. 1999), including T cells (Smith et al. 2003). PDE7A mRNA has been shown to be expressed in multiple tissues including the lung and inflammatory cells (Table 1) (Bloom &Beavo 1996) (Han et al. 1997) (Lugnier 2006). Inhibitors of PDE7 have shown to potentiate the effects of PDE4 inhibitors, suggesting that a combined PDE4:PDE7 inhibitor would be an effective drug.

3. PXS TPI1100: The drug

The relative lack of advancement and the slow pace of innovation to identify new drug products for COPD can be indicative of the complicated nature of this chronic diseases as well as a potential limited number of targets for conventional small molecule drugs and biologics. Moreover, the activity of cytokines, growth factors and chemokines depends on the interaction of these proteins with their cell surface receptors involving large protein-protein interactions or involving interactions between multiple sites on the protein, which could be particularly challenging to disrupt with small molecule inhibitors or biologics (Johnson et al. 2005). To side-step these complications, we have attempted to design an antisense oligonucleotide (AON) based therapy which functions by targeting RNA directly rather than the protein product.

3.1 Antisense oligonucleotides: An overview

Oligonucleotides (ODN) are short polymers of nucleotides that come in various forms, lengths and modifications which can be distinguished into two main groups based on two distinct mechanisms of action; ODN in the first group target RNA and those from the second group target proteins.

RNA-targeting ODN drugs are designed to bind to a specific sequence of a messenger RNA (mRNA) through Watson-Crick base-pairing interactions. Therefore, the site of action of this class of drug is not the protein itself, but rather "upstream" of it, the RNA coding for the protein. The principle of RNA-based therapy is the reduction in the level of a protein through hindrance of its translation. Archetypes of this class of ODN are AON and small interfering RNA (siRNA). AON drugs are single stranded, usually only approximately 20-bases long, which prevent translation of the target RNA via one of two mechanisms. The first mechanism involves the activation of the enzyme RNAse H, which cleaves the RNA moiety of the duplex formed by the binding of the AON drug to its target RNA leading to subsequent reduction in protein synthesis (Stein &Hausen 1969). The second mechanism involves a steric interaction of the AON with the target mRNA that prevents key maturation

steps processes such as splicing and thus preventing translation (Crooke 2008). siRNA drugs share the same mechanism of action to AON, degradation of the protein encoding RNA. However, these drugs are distinct from AON molecules, as they comprise double stranded RNA (varying from 19 to 27 base pair long) (Wu et al. 1998) and induces silencing via the RNA-induced silencing complex (RISC), which is composed of several proteins, including specific RNA-degrading enzymes (Holen et al. 2003).

Similar to conventional small molecules drugs or biologics, the second group of ODN comprises molecules that target proteins directly. Two examples of this group include aptamers and immunostimulatory sequences (ISS). Aptamers comprise either DNA or RNA and typically have a longer chain length (ie, approximately 40 nucleotides) than other ODN. These agents have a specific 3D structure (Ellington &Szostak 1992; Jayasena 1999) that determines their ability to bind specifically to their protein target acting in a similar manner to conventional antibody therapies (Lee et al. 2006). ISS molecules are single stranded, which sequence is enriched with unmethylated cysteine and guanine motifs (CpG) motifs (Vollmer et al. 2004). ISS can mediate immunostimulatory effects following binding to TLR9, a key member of the innate immune system (Agrawal &Kandimalla 2007).

ODN drugs share a relatively common chemical composition that is based on naturally occurring RNA and DNA, and comprises the three elements of nucleotide bases, pentose sugars and linking phosphate groups. In the past decade, medicinal chemistry has allowed significant improvements in the drug-like properties of ODN including the potential to optimize the stability as well as the pharmacologic, pharmacokinetic and toxicologic properties of these molecules. In general, three types of modifications of ODN can be distinguished. The first type of modification, and the one most commonly used, is the replacement of the oxygen atoms of the naturally occurring phosphodiester bond by sulfur groups (phosphorothioate (PS) linkages) to confer stability to the drug molecule. Nucleotide analogs have also been incorporated. For example, adenosine has been replaced with 2-amino-2'-deoxydenosine, which improves binding of the drug to the target and minimizes the potential for bronchospasm and inflammation induced by adenosine (Vollmer et al. 2004). Finally, the sugar moiety can be modified; for example, the addition of a 2'-*O*-methoxyethyl group to the pentose sugar confers stability to the ODN and enhances binding affinity to the target mRNA (Ward 2010).

The AON constituents comprising PXS TPI1100 incorporate two modifications: a modified phosphate backbone and the incorporation of 2-amino-2′-deozyadenosine. These modifications were aimed at improving the binding affinity of the drug to its mRNA target, reduce the immunostimulatory effect of this class of drug, and improve the lung tolerability after administration by the pulmonary route. *In vivo* testing of these molecules by multiple dosing via intratracheal (i.t.) administration in mice demonstrated that the modified chemistry contained in the PXS TPI1100 sequences was far less immunostimulatory than the typical PS-containing AON. Repeated daily i.t. delivery of PS-containing AON at a dose of 2.5 mg/kg induced a 4-fold increase in the recruitment of total cells in bronchoalveolar lavage (BAL) compared to control mice (treated with vehicle) and lung tissue changes as assessed by the presence of moderate (grade 3) perivascular mixed cell infiltrate and severe (grade 4) alveolar inflammation. In contrast, in mice treated with the same dose of AON bearing the modified chemistry no difference in BAL cells (total cells as well as differential cells) as compared to the vehicle group were observed, nor were there any histopathological

changes in the lung following administration of the modified AON demonstrating an overall improved lung tolerability.

3.2 Drug design

The drug candidate, PXS TPI1100, is a 1:1 mixture of two AON, one which targets two isoforms of PDE4 (4B and 4D) and the second AON targeting PDE7A (Fortin et al. 2008). The rationale for developing these new specific and multi-targeted AON is to provide a new class of anti-inflammatory agents that act more broadly on the underlying inflammatory-triad - recruitment, activation and potentiation of processes in chronic respiratory diseases and that is more potent than selective PDE4 inhibitors. Delivery directly to the site of action, the lung, will ensure local deposition of the drug and limited systemic exposure thus reducing potential side effects associated with the systemic delivery (e.g. oral delivery) of PDE4 inhibitors. Lastly, PXS TPI1100 consists of aerosolization of a simple aqueous solution, and does not require any specialized carriers.

AON drugs, while still early in development, possess properties that could be advantageous over classical small molecule drugs (Table 2). First, as a single mRNA strand can be translated into multiple copies of proteins (~5000 copies), there is a clear advantage of "upstream" targeting, that is targeting the mRNA rather than the protein (Popescu 2005). The "upstream" targeting approaching with AON can be achieved irrespectively of the location of the target protein, whether it is inside the cell or outside the cell. AON have the potential to amplify

Potential advantages	• High degree of specificity (primarily for RNA-targeted drugs) • Broad range of potential targets • Ability to modify the properties of the oligonucleotide through chemical modification • Ability to screen efficiently for off-target effects • Absence of hypersensitivity reactions • Relatively short development timelines • Relative ease of formulation for inhaled delivery • Relative ease of formulation of combination products • Relative stability of drug compound and product
Challenges	• Cellular uptake and intracellular release for larger oligonucleotides • Potential immunostimulatory effects • Oligonucleotide stability • Specific systemic toxicological findings
Potential advantages of application in lung disease	• Multi-targeting feasible • Direct delivery to the site of action in the lungs • Cellular uptake and release without additional carrier or formulation technologies • Low systemic exposure

Table 2. Advantages and challenges in the development of antisense oligonucleotides drug candidates.

potency as compared to small molecule drugs which target the protein directly. Furthermore, by targeting the mRNA this method avoids the complications of protein interactions and effects of phosphorylation which can be of concern for PDE inhibitors.

By its very nature, AON are designed to target a specific RNA sequence and this specificity lends an advantage over ICS. As comparison, corticosteroids which are believed to directly regulate between 10 to 100 genes per cell, with a further estimation of many other genes indirectly regulated through interaction of other transcription factors and coactivators by yet unclarified mechanisms (Barnes 2006). In contrast, the inherent specificity of AON for its target avoids the non-selective inhibition nature of steroids. However, AON, as all drugs, have the potential of causing unwanted toxicities or side effects, of which some of these unwanted toxicities can arise because of the inherent capacity of AON to hybridize to RNA. Such toxicities are termed hybridization-dependent and can be subdivided into effects caused by exaggerated pharmacology, i.e. inhibition of the intended target to a degree that produces deleterious effects, and hybridization-dependent effects on unintended RNA targets (off-targets) that happen to be completely or partially complementary to the AON sequence. For the former, with recent advances for the modifications of the AON chemistry to improve binding affinity, as well as improvements for more effective delivery systems, there could be potential risk in designing AON that are too effective. Correct dosing assessment would be imperative. With regard to off-target effects, the use of genomic information databases allows for identification of possible off targets early in the drug discovery process. Any potential off targets can then be monitored both during the preclinical development and safety assessment stage as well as in clinical studies if needed. Along with the hybridization-dependent toxicities, there are also hybridization-independent which are due to interactions between the AON and proteins. The majority of toxicities observed for AON tested to date are hybridization independent and result from AON chemistry or composition of the delivery system and such potential is assessed in animal toxicology studies (Levin et al. 2001).

A further advantage of AON is the common composition and chemical nature of AON allow for an ease in combining two or more AON for a multi-targeted drug, unlike typical combination therapies. Historically, combination therapies have resulted from combining two marketed drugs into a single drug product. In the respiratory space, the combination of a corticosteroid with a long acting β2-adrenergic receptor agonist has been effective at producing billion dollar drugs like Advair (fluticasone/salmeterol), and Symbicort (budesonide/formoterol). Each of the individual components of these drugs had undergone the development process as single entities which were then combined later for a final product. However, the current understanding of various disease systems would suggest the selection and development of drugs that contain at least 2 molecules directed against at least 2 targets from the beginning of the development process.

The rationale for developing these new specific and multi-targeted ODN inhibitors is to provide a new class of anti-inflammatory agents that act more broadly on the underlying inflammatory-triad - recruitment, activation and potentiation of processes in chronic respiratory diseases. Complex diseases require multiple approaches to circumvent the cellular signaling redundancy underlying inflammatory conditions. In an attempt to improve bronchoconstriction and airway hyperresponsiveness in respiratory diseases, drugs have been designed to modulate the immune response by targeting immune

mediators such as cytokines, chemokines or their receptors. It is believed that in order to treat chronic inflammation a single drug directed against multiple targets and pathways would be better at arresting the progression of these respiratory diseases. However, to date there has been limited success with therapies targeting either a single cytokine, chemokine or their receptor highlighting the challenge in treating these complex inflammatory diseases by focusing on a single component or aspect of the inflammation process. Drugs acting on individual molecular targets usually exert unsatisfying therapeutic effects or have severe toxicity or undesired side effects when used in diseases of complicated causes such as in oncology or in inflammatory diseases. One approach to address such limited efficacy and toxicity has been by the development of novel therapies using a mixture of molecules. In oncology for example, a prevailing idea is that inhibiting both cancer cells and cells of the stroma supporting the tumor or blood vessels would gain better results in fighting this disease.

There is a fine balance between specificity and reduced toxicity that can be obtained by targeting more than one cytokine or chemokine or receptor in the immune response without the overwhelming suppression observed with corticosteroids. The era of designing "one target for one disease" has evolved such that the single-target therapy is fading in favor of a multi-targeted approach and the new generation therapies are selected on the basis of their ability to simultaneously inhibit or affect several targets. Through combining two or more molecules which individually have their own target into a single therapeutic product, it may be possible to generate a drug that is potentially more effective, in particular in those patients non-responding to the conventional therapies. In addition, the lower doses could results in less side effects than with broader therapies like corticosteroids. This approach is especially important because of the redundancy of inflammatory pathways indicates the need for AON against multiple genes in one product.

Lastly, PXS TPI1100 consists of aerosolization of a simple aqueous solution, and does not require any specialized carriers unlike many other AON therapies. Indeed, direct administration of low doses to the site of action by inhalation permits AON to efficiently reach and enter the target cells (Figure 2).

3.3 Preclinical pharmacology

In vitro pharmacology studies of the AON candidates of PXS TPI1100 were conducted in both human and animal cell cultures. Results in normal human bronchoepithelial (NHBE) primary cells and a lung epithelial cell line (A549) confirmed the efficacy of PXS TPI1100 at reducing PDE mRNA target knockdown, which is the proposed mechanism of action of the drug. Moreover, in NHBE cells, inhibition of the PDE4B, PDE4D and PDE7A with PXS TPI 1100 resulted with a synergistic effect on the inhibition of IL-8 secretion in response to a stimulus (a mixture of cytokines TNF-α, IL-1β and IFN-γ) compared to when cells were treated with each AON alone (Figure 3). These results and the lack of efficacy of rolipram (small molecule PDE4 inhibitor) on IL-8 confirmed the benefit of PDE4 and PDE7 inhibition. Besides IL-8, cells treated with PXS TPI1100 had an inhibition of the expression and release of other inflammatory mediators (e.g. MCP-1, MMPs). A second model used the lung epithelial cell line, A549, stimulated with the cytokine IL-1β, and again the inhibitory effect of PXS TPI1100 upon the induction of key inflammatory mediators (IL-8, MCP-1) in response to IL-1β was observed.

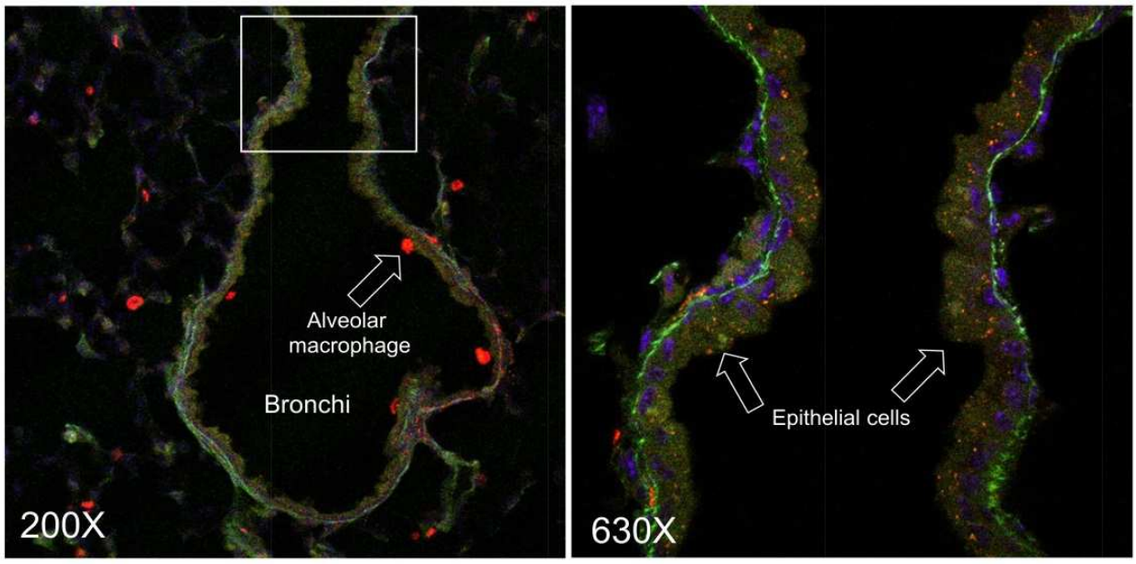

Fig. 2. Intracellular localization of PXS TPI1100 AON constituents in the lung of mice following cigarette smoke exposure. Mice exposed to cigarette smoke were treated intratracheally with a single dose of labelled PXS TPI1100 (a FITC-labeled AON against PDE4B/4D) and a Cy3-labeled AON against PDE7A. Images obtained using a confocal microscope (FITC in green, Cy3 in red and DAPI in blue). Magnification of 200X (left panel) and insert shown at 630X (right panel).

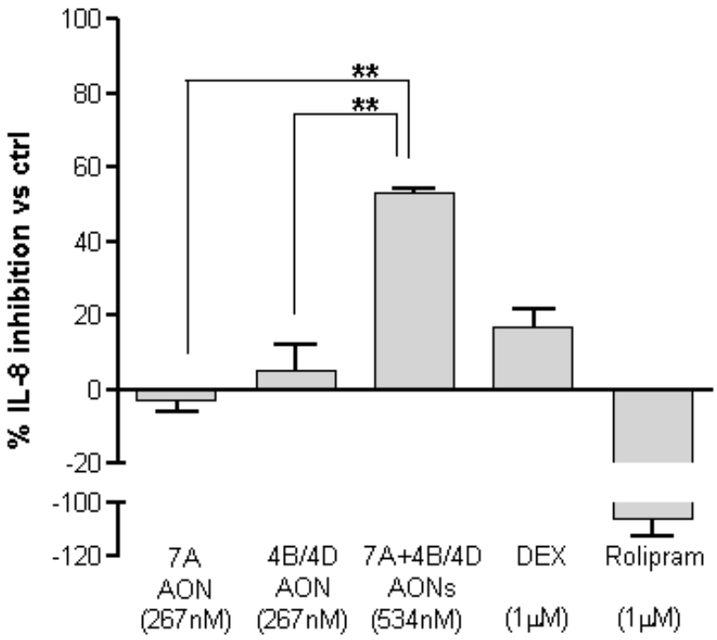

Fig. 3. Activity of PXS TPI1100 in NHBE cells. NHBE cells were treated with the PDE7A or the PDE4B/4D AON alone at indicated concentration or in combination prior to stimulation of the cells (mix of TNF-α, IL-1β and IFN-γ). Inhibition of the three PDE isoforms resulted with a synergistic effect (**$p<0.05$) on IL-8 secretion compared to each AON alone, and a more potent effect than rolipram or dexamethasone (DEX).

In two different *in vivo* models, PXS TPI1100 was shown to reduce the neutrophil influx in the BAL of mice either in response to cigarette smoke or to LPS challenge. Cigarette smoke exposure of laboratory animals reproduces many of the anatomic/physiologic lesions (neutrophilic inflammation, emphysema, small-airway remodeling and pulmonary hypertension) of human COPD (Wright et al. 2008) and has been used for the preclinical assessment of Daxas/Deliresp (Martorana et al. 2005). In this model, mice were exposed to cigarette smoke for 4 consecutive days and treated with PXS TPI1100 every other day (two treatments only) 3 h prior to cigarette smoke exposure. Following repeated smoke exposure, a significant increase (180-fold) recruitment of neutrophils in BAL collected the day after the last smoke exposure was observed compared to mice not exposed to smoke. The percentage of neutrophils in BAL also increased with smoke from 0.8% to 35%. When mice were treated with PXS TPI1100 at 0.1 or 0.4 mg/kg every other day, the smoke-induced neutrophil recruitment was significantly reduced (up to 52% inhibition $p<0.01$) when compared to mice treated with vehicle or a comparable dose of a control AON.

In the second model of acute lung inflammation, mice exposed to LPS (nasal instillation) had a strong inflammatory response with significant increase in neutrophils in BAL. PXS TPI1100 treatment at 1.2 mg/kg (1 h prior to LPS challenge) resulted in a 33% reduction of neutrophil recruitment induced by LPS ($p<0.05$) whereas treatment with the control AON had no effect.

The potency of PXS TPI1100 at reducing the smoke-induced or LPS-induced lung inflammation was compared to the PDE4 inhibitor roflumilast (Daxas). Roflumilast (5 mg/kg, p.o.) given daily 1 h prior to cigarette smoke exposure reduced neutrophil recruitment by only 25% (Fortin et al., 2009). In the LPS model, roflumilast, given once at a dose of 10 mg/kg (~10-fold more than PXS TPI1100) had no effect on the neutrophil influx, whereas at a higher dose of 100 mg/kg (~100-fold that of PXS TPI1100) it reduced neutrophil recruitment by 46% ($p<0.05$). This effective dose of roflumilast exceeds the current clinical dose for Daxas of 500 microgram per adult per day. PXS TPI1100 is continuing its pre-clinical development as a treatment for COPD.

PXS TPI1100 has not yet performed nonclinical drug depositions studies however, from tests with different AON that recognize the same PDE targets yet lacked the modified chemistry backbone we can extrapolate how PXS TPI1100 will behave following pulmonary delivery. In CD-1 mice, following 14 days of daily dosing with AON by inhalation, AON plasma concentrations were not detectable (< LLOQ of 5-10 ng/mL) at all time points for all dose levels. In the lungs, the AON concentrations were dose-related, and there was evidence of accumulation in lungs over the 14 days, based on the higher levels at 24 h after the last dose *vs.* 24 h after the first dose. The systemic exposure was extremely low with only small amounts of AON detectable in the kidneys and liver of high-dose mice (2.5 mg/kg/day), and the levels were similar following the first and last doses. In monkeys, following 14 days of inhalation of AON there were detectable levels of AON in plasma only in a few high-dose animals up to 1 h post dosing on Day 1 while samples from Day 14 were all <LLOQ (Guimond et al. 2008). In the lung of animals on the day after last drug exposure, the AON levels were approximately dose proportional. In kidney and liver, low levels of AON were quantified one day after the last dose and only in high dose animals, demonstrating that similar to mice, the systemic exposure was low. When AON were delivered by slow bolus intravenous (IV) administration in monkey, the highest plasma levels were measured immediately at the first time point after IV

injection (approximately 5 min) and these levels were greatly reduced by 4 h post-dose and near LLOQ by 24 h demonstrating the clearance of AON from the system.

The pharmacokinetics properties following pulmonary delivery has been well characterized (Templin et al. 2000; Ali et al. 2001; Guimond et al. 2008) and confers a significant advantage of AON over small molecule drugs. For example, orally-delivered Daxas/Daliresp has a bioavailabilty of 79%(David 2004) and with an elimination half-life of 14-18 h there is a greater opportunity for this drug to act upon PDE4 outside of the lung and for a long period of time. In comparison, PXS TPI1100 has reduced systemic bioavailability and based on results in mouse lung, the half-life of PXS TPI 1100 has been shown to be relatively short (<5h) suggesting a potentially safer drug that would work locally at the site of action in the lung.

3.4 Clinical experience

To date, PXS TPI1100 has not been dosed in human subjects, however, a review of the current literature on clinical study designs and using the Daxas/Daliresp background as guidance, the projected clinical path for PXS TPI1100 has been defined. Furthermore, reviewing its pharmacology profile, there are potential advantages for PXS TPI1100 which may be manifested in the clinic. In this section, we will first outline some general challenges facing COPD clinical study design, then capture some of the salient points from the Daxas/Daliresp experience in clinical study design.

3.4.1 Challenges in COPD clinical studies

Typically COPD clinical studies measure as a primary outcome lung function by spirometry, either through improvement in postbronchodilator FEV_1 or in cases where assessing the efficacy of nonbronchodilators is preferred, the measure is of the change from baseline in prebronchodilator FEV_1 (Giembycz &Field 2010). As a procedure for detection of airflow obstructions spirometry is a reliable, simple, non-invasive, safe, and non-expensive (Soriano et al. 2009). The test is relatively standardized with most COPD guidelines accepting the threshold to define a positive bronchodilation test as suggested by the Global Initiative for asthma (increase in FEV_1 larger than 12% and 200 mL from the prebronchodilator value) (Bateman et al. 2008) with the variation of suggesting minimum limits of 300 or 400 mL.

Besides the use of these spirometry measures, there have been attempts to determine a relevant easily accessible and rapid assessed biomarker as a measure of improvement. In preclinical pharmacology studies, animal models of neutrophil inflammation are routinely used for efficacy measures in an attempt to mimic the disease state in humans. It is known that in COPD patients the percentage of sputum neutrophils are increased with each GOLD stage, are also raised in COPD exacerbations (Caramori et al. 2003) (Papi et al. 2006), and that neutrophils are involved in the pathogenesis of emphysema through the secretion of proteases and elastases (Cowburn et al. 2008) (Sharafkhaneh et al. 2008). Taken together, these observations would suggest that sputum neutrophils have the potential to be a biomarker predictor of the degree of airflow obstruction, however the reality is far from clear. Reports with small cohorts of patients suggest a relationship between sputum neutrophils measures and FEV_1 (% predicted) (O'Donnell et al. 2004), however a larger cohort study by Singh et al. (Singh et al. 2010) demonstrated that this relationship is only weakly associated. A similar finding was shown with regard to sputum neutrophils

measures and the relationship to health status as defined by the use of the St. Georges Respiratory Questionnaire (SGRQ) (Singh et al. 2010). Furthermore sputum neutrophil measures in the stable state were shown not be predictive of the future rate of exacerbations (Singh et al. 2010). Lastly, no association between sputum neutrophils measures and emphysema or systemic inflammation as measured by serum levels of IL-6, IL-8, C-reactive protein (CRP) and surfactant protein D was observed (Singh et al. 2010). In short, although there is a plausible assumption for the use of sputum neutrophils as a biomarker, there is little validity in using them in face of the current evidence.

In lieu of the identification and validation of a biomarker that could predict the rate of lung function decline in COPD, most COPD clinical trials attempt to measure relevant changes in exacerbations. Exacerbation frequency has been considered to be an important outcome parameter in COPD as it is associated with increase in mortality (Patil et al. 2003) (Fuso et al. 1995). Measuring exacerbations is not without its challenges. It is difficult among studies to find consensus on what is defined as an exacerbation and to gauge the severity of the exacerbation. Symptom-based definitions include use of diaries, while event-based definitions may refer to hospitalizations or use of antibiotics and/or steroids (Miravitlles et al. 2004). Although a systematic literature review of studies reporting exacerbation frequency in COPD patients showed the relationship between increased exacerbation frequency with decreasing lung function to be borderline significance (p=0.053) (Spencer et al. 2004), exacerbations are still considered to be an important parameter in COPD. Exacerbations are more likely to occur in winter and according to current recommendations (Cazzola et al. 2008) studies need to have at least a 12 month follow up to give reliable estimate of exacerbation frequency, which requires the planning of lengthy clinical trials.

The clinical program of PXS TPI1100 has not been initiated yet we expect its design can follow that of other PDE4 inhibitors. An initial Phase 2 study design does not test in COPD patients but rather in allergic asthmatic patients following inhaled allergen challenge (2009). Another AON drug, ASM8 designed specifically for asthma and as such has targets different from PXS TPI1100, has demonstrated clinical efficacy in this allergen challenge model (Gauvreau et al. 2008; Gauvreau 2010) clearly showing the potential for the AON approach. An advantage of this allergen challenge model is that the studies are generally brief in duration and the fall in FEV_1 is a well-recognized response as well as the incorporation of monitoring induced sputum allows for other inflammatory indicators to be measured.

PXS TPI1100 has an advantage in that the clinical studies performed by Daxas/Daliresp can be used as a guide, as the two drugs share a common target. As Daxas/Daliresp was breaking new ground many studies had to be performed and it is plausible to conclude that for other drugs in the same class fewer studies may be required. In all, six phase 3 clinical trials were undertaken with Daxas/Daliresp which have been excellently reviewed by Giembycz and Field (Giembycz &Field 2010). Key aspects of these trials that can be used for PXS TPI1100's clinical development include criteria for patient selection and parameters selected for primary and secondary outcomes. In the phase 3 study named RECORD, patients with moderate-to-severe COPD (postbronchodilator FEV_1 of 30% to 80% predicted and a FEV_1/FVC ratio of less than 70%) were randomized to receive either Daxas/Daliresp at 250 µg or 500 µg or placebo (2:2:1 ratio) for 24 weeks (Rabe et al. 2005). Results showed treated patients experienced improvement in postbronchodilator FEV_1 (Rabe et al. 2005) and a change in SGRQ but this change did not reach clinical significant threshold. Although direct

comparisons between doses were not made, as it seemed that patients receiving the higher dose had better and earlier responses in most outcomes the daily dose of Daxas/Daliresp of 500 μg was then used in two subsequent identical trials (RATIO and OPUS). In these studies the patients had more severe COPD than in the RECORD study (postbronchodilator FEV_1 of 50% or less, FEV_1:FVC ratio of 0.7 or less, or FEV_1 reversibility of 5% or less). Although completed, the results from the OPUS trial have not been published, however results from the RATIO study showed an improvement for the change from baseline for post bronchodilator FEV_1, yet again no effect on the SGRQ (Calverley et al. 2007). A post-hoc analysis of a subgroup of patients with GOLD stage IV disease in the RECORD study showed a significant effect on reduction of exacerbation frequency (Calverley et al. 2007) which then led to the design of two identical studies AURA and HERMES where patients had a diagnosis of clinical COPD (confirmed by postbronchodilator FEV_1/FVC of at least 70%, and a FEV_1 at least 50% of predicted), had symptoms of chronic bronchitis and a history of exacerbations. Patients experienced an improvement in pre- and postbronchodilator FEV_1 and a reduction in exacerbation rate (Calverley et al. 2009) which were independent of LABA use, but no differences in mortality or C-reactive protein levels.

Taken together, the Daxas/Daliresp studies clearly show effects in patients with GOLD stage IV disease, with focus on measuring flow rates and exacerbation reduction as parameter outcomes. The clinical program for PXS TPI1100 can use this information in designing studies so as to sharply define the patient population at the onset and include the key primary outcomes as success measures.

As ICS and LABA have been shown to be more effective at improving lung function, health status and reducing COPD exacerbations when combined than when used individually (Calverley et al. 2007) the effect of combining Daxas/Daliresp with either the long-acting β2-agonist salmeterol (EOS study) or the long-acting inhaled antimuscarinic tiotroprium (HELIOS study) was studied in patients with less severely reduced lung function as compared to the previous studies. Results showed that the pre- and postbronchodilator FEV_1 improved in patients treated with Daxas/Daliresp versus placebo when combined with either LABA or LAMA (Fabbri et al. 2009). PXS TPI1100 can be expected to also function in combination with LABA, similar to that demonstrated by Daxas/Daliresp and could potentially replace ICS.

As with any drug, adverse events to Daxas/Daliresp were reported which included weight loss, diarrhea, nausea, headache, influenza and nasopharyngitis as well as certain cancers such as lung and prostate (Giembycz &Field 2010). There was a greater risk of discontinuation of therapy within the first 12 weeks of treatment for those patients taking Daxas/Daliresp than placebo although by the end of the studies, similar numbers of patients withdrew in both groups. In the Daxas/Daliresp treated groups, the most common reason for withdrawal were the gastrointestinal adverse events or headache (Giembycz &Field 2010).

There are aspects of PXS TPI1100 which may lend itself advantages over Daxas/Daliresp. Firstly, as PXS TPI1100 is administered via inhalation, it is delivered directly to the intended site of action of the lung (Ali et al. 2001; Duan et al. 2005; Gauvreau et al. 2008; Guimond et al. 2008) where the drug can enter target cells directly (Zhang et al. 2004; Griesenbach et al. 2006) thus potentially reducing total dose as compared to orally-available treatments. A further advantage of pulmonary administration of AON is that they are principally

metabolized in the lung with very limited systemic delivery after inhalation (Templin et al. 2000; Ali et al. 2001; Guimond et al. 2008) which leads to reduced systemic bioavailability of the drug. In comparison to Daxas/Daliresp, which is delivered orally and has a high level of bioavailability, the projected low systemic bioavailability of PXS TPI1100 may limit adverse events associated with PDE4 inhibitors, namely the gastrointestinal and neurological side effects. Another consideration is the projected brief half-life of PXS TPI1100. Based on the mouse lung, the half-life of PXS TPI1100 has been shown to be relatively short (<5h), although it is reassuring that this short tissue half-life does not appear to affect the efficacy of the drug as every-other day dosing of PXS TPI1100 in the smoking mouse model was highly effective. Reconciling the short half-life with longer term efficacy may be a reflection of the mechanism of action of the drug, suggesting that inhibition of PDE mRNA has longer term consequences on downstream effects including limitation of inflammatory responses.

Besides a projected favorable safety profile resulting from low systemic bioavailability, PXS TPI1100 can be expected to avoid the toxicity associated with the broader approach of anti-inflammatories such as ICS by specifically targeting PDE.

4. Conclusions

PXS TPI1100 faces challenges, in part of being the first respirable antisense drug product in COPD. As COPD is a chronic disease, it can be expected that patients will be dosed for years. The long-term effects of this drug class have never before been studied. In addition, in pulmonary/respiratory diseases, there is a risk that administration of therapeutic nucleic acids may lead to immune stimulation, inflammation and possibly hypersensitivity and bronchoconstriction of the airways. Except for the latter, these risks are not specific to the lung as they have been observed with other routes of administration. As with any novel inhaled medication, local tolerability and the absence of long-term effects following chronic dosing will require careful evaluation as drug candidate progresses through the later stages of development. The publicly available toxicological data on inhaled AON are not extensive (Guimond et al. 2008), and therefore deriving definitive conclusions on toxicology at this time is not possible. The phase 2a studies that have been performed until now have not shown any of this potential toxicity but longer term studies are needed to confirm these results. Furthermore, to date AON have been delivered via inhalation of a nebulisate to asthma patients (Gauvreau et al. 2008; Gauvreau 2010), but not to COPD patients who have severely decreased FEV_1. How well this patient cohort inhales the nebulisate would need to be determined. The range of delivery devices (including newer portable soft-mist inhalers) have increased and permit liquid aerosols to be targeted more effectively to the specific airways of interest (upper or lower airways), improve ease of use by patients and would be expected to improve compliance to therapy. In contrast, the particle processing and formulation of AON for delivery in dry powder inhalers or pressurized metered dose inhalers, which are most commonly used by COPD patients has, however, proven to be significantly more challenging than that of liquid aerosols.

Another challenge facing PXS TPI1100 is the selection of its targets PDE4B/D and PDE7A. While the success of Daxas/Daliresp demonstrates the effectiveness of targeting PDE4 in COPD, to date there is less corrobative clinical evidence for the efficacy of targeting PDE7A isoform. The success or failure of a specific drug development program is determined by a range of different factors, which includes the clinical relevance of the selected drug target.

One early pioneer in the respiratory field was the AON drug EPI 2010 (Epigenesis) targeting the promoter region of the adenosine A1 receptor. Although demonstrating efficacy *in vitro* and in animal models (Ball et al. 2004), EPI 2010 failed in later clinical studies to demonstrate efficacy to improve lung function in asthmatics. With the more recent understanding of the role the different adenosine receptors have in asthma (Brown et al. 2008), it could be argued that the absence of clinical efficacy for EPI 2010 could either be a result of targeting the wrong adenosine receptor or perhaps the need to combine it with other adenosine receptor inhibitors. Similarly, early preclinical efficacy and effect on biomarkers in a phase 1 study with AIR-645 (AON targeting IL-4/IL-13Rα Altair/Isis) met with apparent insufficient efficacy on lung function in phase 2 study (personal communication). This may perhaps be attributed to the target selection as other non-ODN drugs targeting these receptors have also had limited success in clinical trials. As mentioned, although few PDE7 inhibitors have been tested in clinical studies, our preclinical pharmacology results indicate a clear benefit in targeting this PDE isoform along with the PDE4. There is growing acceptance that multi-targeted approaches may provide significant therapeutic advantages, as demonstrated by the issuance of new guidance on drug combinations by the Food and Drug Administration.

There is a clear need for innovative products with novel mechanisms of action to complement today's inhaled products particularly for severe patients who seem resistant to current therapeutic interventions. In spite of many attempts, success in these respiratory indications has been modest, at most. This may reflect the challenge of delivering the therapies to the site of action (lung) or more importantly the complexity of these diseases. PXS TPI1100 belongs to a new class of therapeutics that is poised to expand in the upcoming decades because of its advantages, especially with lung administration. Outstanding challenges for PXS TPI1100 remain the need to establish long term safety and tolerability data as well as commence clinical efficacy. The future remains very promising for this novel drug.

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6. References

(2009). Study To Evaluate GSK256066 In Subjects With Mild Bronchial Asthma.

Agrawal, S. and Kandimalla, E. R. (2007). Synthetic agonists of Toll-like receptors 7, 8 and 9 *Biochem. Soci. Trans.* Vol.35 No.(6): pp.1461-1467

Ali, S., Leonard, S. A., Kukoly, C. A., James Metzger, W., Wooles, W. R., McGinty, J. F., Tanaka, M., Sandrasagra, A. and Nyce, J. W. (2001). Absorption, Distribution, Metabolism, and Excretion of a Respirable Antisense Oligonucleotide for Asthma *Am. J. Respir. Crit. Care Med.* Vol.163 No.(4): pp.989-993

Ariga, M., Neitzert, B., Nakae, S., Mottin, G., Bertrand, C., Pruniaux, M. P., Jin, S. L. C. and Conti, M. (2004). Nonredundant Function of Phosphodiesterases 4D and 4B in Neutrophil Recruitment to the Site of Inflammation *J Immunol* Vol.173 7531-7538

Ball, H. A., Van Scott, M. R. and Robinson, C. B. (2004). Sense and antisense: therapeutic potential of oligonucleotides and interference RNA in asthma and allergic disorders *Clin Rev Allergy Immunol* Vol.27 No.(3): pp.207-217

Barnes, P. J. (2006). How corticosteroids control inflammation: Quintiles Prize Lecture 2005 *Br J Pharmacol* Vol.148 No.(3): pp.245-254

Bateman, E. D., Hurd, S. S., Barnes, P. J., Bousquet, J., Drazen, J. M., FitzGerald, M., Gibson, P., Ohta, K., O'Byrne, P., Pedersen, S. E., Pizzichini, E., Sullivan, S. D., Wenzel, S. E. and Zar, H. J. (2008). Global strategy for asthma management and prevention: GINA executive summary *Eur. Respir. J.* Vol.31 No.(1): pp.143-178

Bender, A. T. and Beavo, J. A. (2006). Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use *Pharmacol Rev* Vol.58 No.(3): pp.488-520

Bloom, T. J. and Beavo, J. A. (1996). Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants *Proc Natl Acad Sci U S A* Vol.93 No.(24): pp.14188-14192

Brown, R. A., Spina, D. and Page, C. P. (2008). Adenosine receptors and asthma *Br J Pharmacol* Vol.153 Suppl 1 S446-456

Calverley, P. M., Anderson, J. A., Celli, B., Ferguson, G. T., Jenkins, C., Jones, P. W., Yates, J. C. and Vestbo, J. (2007). Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease *N Engl J Med* Vol.356 No.(8): pp.775-789

Calverley, P. M., Rabe, K. F., Goehring, U. M., Kristiansen, S., Fabbri, L. M. and Martinez, F. J. (2009). Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials *Lancet* Vol.374 No.(9691): pp.685-694

Calverley, P. M. A., Sanchez-Toril, F., McIvor, A., Teichmann, P., Bredenbroeker, D. and Fabbri, L. M. (2007). Effect of One Year Treatment with Roflumilast in Severe Chronic Obstructive Pulmonary Disease *Am. J. Respir. Crit. Care Med.* Vol.200610-201563OC

Caramori, G., Romagnoli, M., Casolari, P., Bellettato, C., Casoni, G., Boschetto, P., Chung, K. F., Barnes, P. J., Adcock, I. M., Ciaccia, A., Fabbri, L. M. and Papi, A. (2003). Nuclear localisation of p65 in sputum macrophages but not in sputum neutrophils during COPD exacerbations *Thorax* Vol.58 No.(4): pp.348-351

Cazzola, M., MacNee, W., Martinez, F. J., Rabe, K. F., Franciosi, L. G., Barnes, P. J., Brusasco, V., Burge, P. S., Calverley, P. M. A., Celli, B. R., Jones, P. W., Mahler, D. A., Make, B., Miravitlles, M., Page, C. P., Palange, P., Parr, D., Pistolesi, M., Rennard, S. I., Rutten-van Molken, M. P., Stockley, R., Sullivan, S. D., Wedzicha, J. A., Wouters, E. F. and on behalf of the American Thoracic Society/European Respiratory Society Task Force on outcomes of, C. (2008). Outcomes for COPD pharmacological trials: from lung function to biomarkers *Eur Respir J* Vol.31 No.(2): pp.416-469

Cowburn, A. S., Condliffe, A. M., Farahi, N., Summers, C. and Chilvers, E. R. (2008). Advances in neutrophil biology: clinical implications *Chest* Vol.134 606-612 0012-3692 (Print)

Crooke, S. T., Ed. (2008). *Antisense Drug Technology: Principles, Strategies and Applications*, CRC Press, Boca Raton FL

David, M., Zech. K., Seiberling, M., Weimar, C. and Bethke, TD. (2004). Roflumilast, a novel, oral, selective PDE4 inhibitor, shows high absolute bioavailabilty. *Journal of Allergy and Clinical Immunology* Vol.113 No.(suppl 2): pp.S220-221

Down, G., Siederer, S., Lim, S. and Daley-Yates, P. (2006). Clinical pharmacology of Cilomilast *Clin Pharmacokinet* Vol.45 No.(3): pp.217-233

Duan, W., Chan, J. H., McKay, K., Crosby, J. R., Choo, H. H., Leung, B. P., Karras, J. G. and Wong, W. S. (2005). Inhaled p38alpha mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice *Am. J. Respir. Crit. Care Med.* Vol.171 571-578 1073-449X (Print)

Ellington, A. D. and Szostak, J. W. (1992). Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures *Nature* Vol.355 No.(6363): pp.850-852

Ernst, P., Gonzalez, A. V., Brassard, P. and Suissa, S. (2007). Inhaled corticosteroid use in chronic obstructive pulmonary disease and the risk of hospitalization for pneumonia *Am J Respir Crit Care Med* Vol.176 No.(2): pp.162-166

Fabbri, L. M., Calverley, P. M., Izquierdo-Alonso, J. L., Bundschuh, D. S., Brose, M., Martinez, F. J. and Rabe, K. F. (2009). Roflumilast in moderate-to-severe chronic obstructive pulmonary disease treated with longacting bronchodilators: two randomised clinical trials *Lancet* Vol.374 No.(9691): pp.695-703

Fortin, M., Higgins, M., Aubé, P., Séguin, S., Moktefi, K., Paquet, L. and Ferrari, N. (2008). TPI 1100: An inhaled antisense oligonucleotide (AON) against phosphodiesterase (PDE) 4 and 7 with significant anti-inflammatory effects in a mouse model for COPD [abstract]. Am. J. Respir. Crit. Care Med. 177: A653.

Fuso, L., Incalzi, R. A., Pistelli, R., Muzzolon, R., Valente, S., Pagliari, G., Gliozzi, F. and Ciappi, G. (1995). Predicting mortality of patients hospitalized for acutely exacerbated chronic obstructive pulmonary disease *Am J Med* Vol.98 No.(3): pp.272-277

Gauvreau, G. M., Boulet, L. P., Cockcroft, D. W., Baatjes, A., Cote, J., Deschesnes, F., Davis, B., Strinich, T., Howie, K., Duong, M., Watson, R. M., Renzi, P. M. and O'Byrne, P. M. (2008). Antisense Therapy against CCR3 and the Common Beta Chain Attenuates Allergen-induced Eosinophilic Responses *Am. J. Respir. Crit. Care Med.* Vol.177 952-958

Gauvreau, G. M., Pageau, R., Seguin, R., Carballo, D., D'Anjou, H., Campbell, H., Watson, R., Parry-Billings, M., Killian, K., and Renzi, P.M. (2010). Efficacy of increasing doses of TPI ASM8 on allergen inhalation challenges in asthmatics. *American Thoracic Society*New Orleans

Giembycz, M. A. and Field, S. K. (2010). Roflumilast: first phosphodiesterase 4 inhibitor approved for treatment of COPD. *Drug Des. Devel. Ther.* Vol.4 147-158

Gold, P. M. (2009). The 2007 GOLD Guidelines: a comprehensive care framework *Respir Care* Vol.54 No.(8): pp.1040-1049

Griesenbach, U., Kitson, C., Escudero Garcia, S., Farley, R., Singh, C., Somerton, L., Painter, H., Smith, R. L., Gill, D. R., Hyde, S. C., Chow, Y. H., Hu, J., Gray, M., Edbrooke, M., Ogilvie, V., MacGregor, G., Scheule, R. K., Cheng, S. H., Caplen, N. J. and Alton, E. W. (2006). Inefficient cationic lipid-mediated siRNA and antisense oligonucleotide transfer to airway epithelial cells in vivo *Respir. Res.* Vol.7 26 1465-993X (Electronic)

Guimond, A., Viau, E., Aube, P., Renzi, P. M., Paquet, L. and Ferrari, N. (2008). Advantageous toxicity profile of inhaled antisense oligonucleotides following chronic dosing in non-human primates *Pulm. Pharmacol. Ther.* Vol.21 845-854 1094-5539 (Print)

Han, P., Zhu, X. and Michaeli, T. (1997). Alternative splicing of the high affinity cAMP-specific phosphodiesterase (PDE7A) mRNA in human skeletal muscle and heart *J Biol Chem* Vol.272 No.(26): pp.16152-16157

Hogg, J. C., Chu, F., Utokaparch, S., Woods, R., Elliott, W. M., Buzatu, L., Cherniack, R. M., Rogers, R. M., Sciurba, F. C., Coxson, H. O. and Pare, P. D. (2004). The nature of small-airway obstruction in chronic obstructive pulmonary disease *N Engl J Med* Vol.350 No.(26): pp.2645-2653

Holen, T., Amarzguioui, M., Babaie, E. and Prydz, H. (2003). Similar behaviour of single-strand and double-strand siRNAs suggests they act through a common RNAi pathway *Nucleic Acids Res.* Vol.31 No.(9): pp.2401-2407

Hurst, J. R., Vestbo, J., Anzueto, A., Locantore, N., Mullerova, H., Tal-Singer, R., Miller, B., Lomas, D. A., Agusti, A., Macnee, W., Calverley, P., Rennard, S., Wouters, E. F. and Wedzicha, J. A. (2010). Susceptibility to exacerbation in chronic obstructive pulmonary disease *N Engl J Med* Vol.363 No.(12): pp.1128-1138

Jayasena, S. D. (1999). Aptamers: An Emerging Class of Molecules That Rival Antibodies in Diagnostics *Clin. Chem.* Vol.45 No.(9): pp.1628-1650

Jin, S. L. C. and Conti, M. (2002). Induction of the cyclic nucleotide phosphodiesterase PDE4B is essential for LPS-activated TNF-alpha responses *PNAS* Vol.99 No.(11): pp.7628-7633

Jin, S. L. C., Lan, L., Zoudilova, M. and Conti, M. (2005). Specific Role of Phosphodiesterase 4B in Lipopolysaccharide-Induced Signaling in Mouse Macrophages *J Immunol* Vol.175 1523-1531

Johnson, Z., Schwarz, M., Power, C. A., Wells, T. N. C. and Proudfoot, A. E. I. (2005). Multi-faceted strategies to combat disease by interference with the chemokine system *Trends Immunol.* Vol.26 No.(5): pp.268-274

Lee, J. F., Stovall, G. M. and Ellington, A. D. (2006). Aptamer therapeutics advance *Curr. Opin. Chem. Biol.* Vol.10 No.(3): pp.282-289

Levin, A. A., Henry, S. P., Monteith, D. K. and Templin, M. (2001). Toxicity of antisense oligonucleotides. Antisense Drug Technology: Principles, Strategies, and Applications S. T. Crooke. New York, Marcel Dekker, Inc.: 201-267.

Li L, Yee C and JA., B. (1999). CD3- and CD28-dependent induction of PDE7 required for T cell activation. *Science.* Vol.283 No.(5403): pp.848-851

Lugnier, C. (2006). Cyclic nucleotide phosphodiesterase (PDE) superfamily: A new target for the development of specific therapeutic agents *Pharmacology & Therapeutics* Vol.109 No.(3): pp.366-398

Martorana, P. A., Beume, R., Lucattelli, M., Wollin, L. and Lungarella, G. (2005). Roflumilast Fully Prevents Emphysema in Mice Chronically Exposed to Cigarette Smoke *Am. J. Respir. Crit. Care Med.* Vol.172 No.(7): pp.848-853

Mehats, C., Jin, S. L. C., Wahlstrom, J. A. N., Law, E., Umetsu, D. T. and Conti, M. (2003). PDE4D plays a critical role in the control of airway smooth muscle contraction *FASEB J.* Vol.17 No.(13): pp.1831-1841

Miravitlles, M., Ferrer, M., Pont, A., Zalacain, R., Alvarez-Sala, J. L., Masa, F., Verea, H., Murio, C., Ros, F. and Vidal, R. (2004). Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study *Thorax* Vol.59 No.(5): pp.387-395

O'Donnell, R. A., Peebles, C., Ward, J. A., Daraker, A., Angco, G., Broberg, P., Pierrou, S., Lund, J., Holgate, S. T., Davies, D. E., Delany, D. J., Wilson, S. J. and Djukanovic, R. (2004). Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD *Thorax* Vol.59 No.(10): pp.837-842

Papi, A., Bellettato, C. M., Braccioni, F., Romagnoli, M., Casolari, P., Caramori, G., Fabbri, L. M. and Johnston, S. L. (2006). Infections and Airway Inflammation in Chronic Obstructive Pulmonary Disease Severe Exacerbations *Am. J. Respir. Crit. Care Med.* Vol.173 No.(10): pp.1114-1121

Patil, S. P., Krishnan, J. A., Lechtzin, N. and Diette, G. B. (2003). In-hospital mortality following acute exacerbations of chronic obstructive pulmonary disease *Arch Intern Med* Vol.163 No.(10): pp.1180-1186

Popescu, F.-D. (2005). Antisense- and RNA interference-based therapeutic strategies in allergy *J. Cell. Mol. Med.* Vol.9 No.(4): pp.840-853

Rabe, K. F., Bateman, E. D., O'Donnell, D., Witte, S., Bredenbroker, D. and Bethke, T. D. (2005). Roflumilast-an oral anti-inflammatory treatment for chronic obstructive pulmonary disease: a randomised controlled trial *Lancet* Vol.366 563-571 1474-547X (Electronic)

Sharafkhaneh, A., Hanania, N. A. and Kim, V. (2008). Pathogenesis of emphysema: from the bench to the bedside *Proc Am Thorac Soc* Vol.5 No.(4): pp.475-477

Singh, D., Edwards, L., Tal-Singer, R. and Rennard, S. (2010). Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study *Respir Res* Vol.11 77

Smith, S. J., Brookes-Fazakerley, S., Donnelly, L. E., Barnes, P. J., Barnette, M. S. and Giembycz, M. A. (2003). Ubiquitous expression of phosphodiesterase 7A in human proinflammatory and immune cells *Am. J. Physiol. Lung Cell Mol. Physiol.* Vol.284 L279-289

Soriano, J. B., Zielinski, J. and Price, D. (2009). Screening for and early detection of chronic obstructive pulmonary disease *Lancet* Vol.374 No.(9691): pp.721-732

Spencer, S., Calverley, P. M., Burge, P. S. and Jones, P. W. (2004). Impact of preventing exacerbations on deterioration of health status in COPD *Eur Respir J* Vol.23 No.(5): pp.698-702

Stein, H. and Hausen, P. (1969). Enzyme from calf thymus degrading the RNA moiety of DNA-RNA Hybrids: effect on DNA-dependent RNA polymerase *Science* Vol.166 No.(903): pp.393-395

Tashkin, D. P., Celli, B., Senn, S., Burkhart, D., Kesten, S., Menjoge, S. and Decramer, M. (2008). A 4-year trial of tiotropium in chronic obstructive pulmonary disease *N Engl J Med* Vol.359 No.(15): pp.1543-1554

Templin, M., Levin, A., Graham, M., Aberg, P., Axelsson, B., Butler, M., Geary, R. and Bennett, C. (2000). Pharmacokinetic and toxicity profile of a phosphorothioate oligonucleotide following inhalation delivery to lung in mice. *Antisense Nucleic Acid Drug Dev.* Vol.10 No.(5): pp.359-368

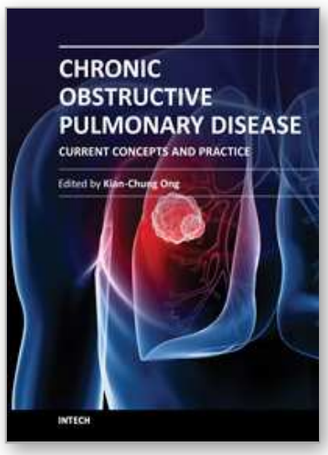
Vollmer, J., Weeratna, R., Payette, P., Jurk, M., Schetter, C., Laucht, M., Wader, T., Tluk, S., Liu, M., Davis, H. L. and Krieg, A. M. (2004). Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities *Eur. J. Immunol.* Vol.34 No.(1): pp.251-262 1521-4141

Ward, P. A. (2010). Oxidative stress: acute and progressive lung injury *Annals of the New York Academy of Sciences* Vol.1203 No.(1): pp.53-59

Wright, J. L., Cosio, M. G. and Churg, A. (2008). Animal Models of Chronic Obstructive Pulmonary Disease *Am J Physiol Lung Cell Mol Physiol* Vol.90200.92008

Wu, H., MacLeod, A. R., Lima, W. F. and Crooke, S. T. (1998). Identification and partial purification of human double strand RNase activity. A novel terminating mechanism for oligoribonucleotide antisense drugs *J. Biol. Chem.* Vol.273 No.(5): pp.2532-2542

Zhang, X., Shan, P., Jiang, D., Noble, P. W., Abraham, N. G., Kappas, A. and Lee, P. J. (2004). Small Interfering RNA Targeting Heme Oxygenase-1 Enhances Ischemia-Reperfusion-induced Lung Apoptosis *J. Biol. Chem.* Vol.279 No.(11): pp.10677-10684



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A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.

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Phone: +385 (51) 770 447
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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