

**OLIGONUCLEOTIDE INHIBITORS
WITH CHIMERIC BACKBONE AND 2-AMINO-2'-DEOXYADENOSINE**

CROSS REFERENCES TO RELATED APPLICATIONS

- 5 This application claims priority from U.S. Provisional Patent Application No. 61/446,144 filed on February 24, 2011.

FIELD OF THE INVENTION

- 10 The present invention relates to oligonucleotides useful in the treatment of inflammation and respiratory diseases, and specifically to oligonucleotide inhibitors of phosphodiesterase expression.

BACKGROUND OF THE INVENTION

- 15 The alveolar and airway epithelium is recognized as a dynamic barrier that plays an important role in regulating inflammatory and metabolic responses to oxidative stress, sepsis, endotoxemia, and other critical illnesses in the lung. The respiratory epithelium, in particular, is a primary target of inflammatory conditions/infections at the epithelial-blood interface, and is itself capable of amplifying an inflammatory signal by recruiting inflammatory cells and
20 producing inflammatory mediators.

Chronic Obstructive Pulmonary Disease (COPD) is one example of an inflammatory airway and alveolar disease where persistent upregulation of inflammation is thought to play a role. Inflammation in COPD is characterized by increased infiltration of neutrophils, CD8 positive lymphocytes, and

macrophages into the airways. Neutrophils and macrophages play an important role in the pathogenesis of airway inflammation in COPD because of their ability to release a number of mediators including elastase, metalloproteases, and oxygen radicals that promote tissue inflammation and damage. It has been suggested that inflammatory cell accumulation in the airways of patients with COPD is driven by increased release of pro-inflammatory cytokines and of chemokines that attract the inflammatory cells into the airways, activate them and maintain their presence. The cells that are present also release enzymes (like metalloproteases) and oxygen radicals which have a negative effect on tissue and perpetuate the disease. A vast array of pro-inflammatory cytokines and chemokines has been shown to be increased within the lungs of patients with COPD. Among them, an important role is played by tumor necrosis factor alpha (TNF-alpha), granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 8 (IL-8), which are increased in the airways of patients with COPD.

Other examples of respiratory diseases where inflammation seems to play a role include: asthma, eosinophilic cough, bronchitis, cystic fibrosis, pulmonary hypertension, acute respiratory distress syndrome (ARDS), acute and chronic rejection of lung allograft, sarcoidosis, pulmonary fibrosis, rhinitis and sinusitis.

Asthma is defined by airway inflammation, reversible obstruction and airway hyperresponsiveness. In this disease the inflammatory cells that are involved are predominantly eosinophils, T lymphocytes and mast cells, although neutrophils and macrophages may also be important. A vast array of cytokines and chemokines have been shown to be increased in the airways and play a role in the pathophysiology of this disease by promoting inflammation, obstruction and hyperresponsiveness.

Eosinophilic cough is characterized by chronic cough and the presence of inflammatory cells, mostly eosinophils, within the airways of patients in the absence of airway obstruction or hyperresponsiveness. Several cytokines and

chemokines are increased in this disease, although they are mostly eosinophil directed. Eosinophils are recruited and activated within the airways and potentially release enzymes and oxygen radicals that play a role in the perpetuation of inflammation and cough.

- 5 Acute bronchitis is an acute disease that occurs during an infection or irritating event for example by pollution, dust, gas or chemicals, of the lower airways. Chronic bronchitis is defined by the presence of cough and phlegm production on most days for at least 3 months of the year, for 2 years. One can also find during acute or chronic bronchitis within the airways inflammatory cells,
10 mostly neutrophils, with a broad array of chemokines and cytokines. These mediators are thought to play a role in the inflammation, symptoms and mucus production that occur during these diseases.

- Lung transplantation is performed in patients with end stage lung disease. Acute and more importantly chronic allograft rejection occur when the
15 inflammatory cells of our body, lymphocytes, do not recognize the donor organ as "self". Inflammatory cells are recruited by chemokines and cytokines and release a vast array of enzymes that lead to tissue destruction and in the case of chronic rejection a disease called bronchiolitis obliterans.

- Sarcoidosis is a disease of unknown cause where chronic non-caseating
20 granulomas occur within tissue. The lung is the organ most commonly affected. Lung bronchoalveolar lavage shows an increase in mostly lymphocytes, macrophages and sometimes neutrophils and eosinophils. These cells are also recruited and activated by cytokines and chemokines and are thought to be involved in the pathogenesis of the disease.

- 25 Pulmonary fibrosis is a disease of lung tissue characterized by progressive and chronic fibrosis (scarring) which will lead to chronic respiratory insufficiency. Different types and causes of pulmonary fibrosis exist but all are characterized by inflammatory cell influx and persistence, activation and proliferation of fibroblasts with collagen deposition in lung tissue. These

events seem related to the release of cytokines and chemokines within lung tissue.

Acute rhinitis is an acute disease that occurs during an infection or irritating event, for example, by pollution, dust, gas or chemicals, of the nose or upper
5 airways. Chronic rhinitis is defined by the presence of a constant chronic runny nose, nasal congestion, sneezing and pruritis. One can also find within the upper airways during acute or chronic rhinitis inflammatory cells with a broad array of chemokines and cytokines. These mediators are thought to play a role in the inflammation, symptoms and mucus production that occur
10 during these diseases.

Acute sinusitis is an acute, usually infectious disease of the sinuses characterized by nasal congestion, runny, purulent phlegm, headache or sinus pain, with or without fever. Chronic sinusitis is defined by the persistence for more than 6 months of the symptoms of acute sinusitis. One
15 can also find during acute or chronic sinusitis within the upper airways and sinuses inflammatory cells with a broad array of chemokines and cytokines. These mediators are thought to play a role in the inflammation, symptoms and phlegm production that occur during these diseases.

There is a growing body of evidence suggesting an intimate link between
20 inflammation and neoplastic diseases. The tumor microenvironment is shaped by cells entering it, and their functions reflect the local conditions. Successive changes occurring at the tumor site during tumor progression resemble chronic inflammation. This chronic inflammatory reaction seems to be largely orchestrated by the tumor, and it seems to promote tumor survival.
25 It has become evident that early and persistent inflammatory responses observed in or around developing neoplasms regulates many aspects of tumour development (matrix remodelling, angiogenesis, malignant potential) by providing diverse mediators implicated in maintaining tissue homeostasis, e.g., soluble growth and survival factors, matrix remodelling enzymes,
30 reactive oxygen species and other bioactive molecules.

As described above, these inflammatory respiratory diseases or diseases in which inflammation plays a critical role are all characterized by the presence of mediators that recruit and activate different inflammatory cells which release enzymes or oxygen radicals causing symptoms, the persistence of inflammation and when chronic, destruction or disruption of normal tissue.

A therapeutic approach that would decrease pro-inflammatory cytokine and chemokine release by a vast array of cells while having a reduced effect on the release of anti-inflammatory mediators or enzymes may have an advantage over current therapies for inflammatory respiratory diseases or any other systemic inflammatory disease.

The cyclic nucleotides cAMP and cGMP are ubiquitous second messengers participating in signaling transduction pathways and mechanisms. Both cAMP and cGMP are formed from their respective triphosphates (ATP and GTP) by the catalytic activity of adenylyl (adenylate) or guanylyl (guanylate) cyclase. Inactivation of cAMP/cGMP is achieved by hydrolytic cleavage of the 3'-phosphodiester bond catalyzed by the cyclic-nucleotide-dependent phosphodiesterases (PDEs), resulting in the formation of the corresponding, inactive 5'-monophosphate. It has been shown that the inflammatory response and its progression is exquisitely sensitive to modulations in the steady-state levels of cyclic nucleotides, where target cells for their effects extend beyond immune cells to include accessory cells, such as airway smooth muscle, epithelial and endothelial cells, and neurons. The cyclic nucleotide PDEs are a large, growing multigene family, comprising at least 11 families of PDE enzymes. The profile of selective and nonselective PDE inhibitors in vitro and in vivo, therefore, suggests a potential therapeutic utility as antidepressants, antiproliferative, immunomodulatory, tocolytics, inotropes/chronotropes, and cytoprotective agents. Use of antisense oligonucleotides ("AONs") directed against PDE are described in co-owned WO 05/030787 and WO 07/134451.

For potential clinical uses, oligonucleotides should exhibit stability against degradation by serum and cellular nucleases, show low non-specific binding

to serum and cell proteins, exhibit enhanced recognition of the target mRNA sequence, demonstrate cell-membrane permeability and elicited cellular nucleases when complexed with complementary mRNA. It is well documented that oligonucleotides containing natural sugars (D-ribose and D-2-
5 deoxyribose) and phosphodiester (PO) linkages are rapidly degraded by serum and intracellular nucleases, which limit their utility as effective therapeutic agents. Chemical strategic modifications have been described for oligonucleotides in order to improve their stability and efficacy as therapeutic agents. The main chemical changes included, modification of the sugar
10 moiety, the base moiety, and/or modification or replacement of the internucleotide phosphodiester linkage. To date the most widely studied analogues are the phosphorothioate (PS) oligodeoxynucleotides, in which one of the non-bridging oxygen atoms in the phosphodiester backbone is replaced with a sulfur.

15 For example, some PO linkages within immunostimulatory sequences such as CpG-containing oligonucleotides have been replaced with PS linkages to increase the stability of the oligonucleotide. However, generally PO linkages within the CpG motif are required and therefore a mixed PO/PS backbone is needed for maximal immunostimulatory activity of the CpG-containing
20 oligonucleotides.

There are examples of antisense oligonucleotides with PO/PS linkages and incorporation of FANA modifications. However, such modifications can reduce the activity of the antisense oligonucleotide, as described in co-owned WO09/137912.

25 Other modifications to oligonucleotides include the replacement of adenosine residues with 2-amino-2'-deoxyadenosine (DAP), as described in co-owned WO 03/004511.

It would be desirable to have improved oligonucleotides directed against PDEs for use in treating inflammation and respiratory diseases.

SUMMARY OF THE INVENTION

In an aspect, there is provided an oligonucleotide directed against a target Phosphodiesterase (PDE), wherein the oligonucleotide is capable of hybridizing to at least a portion of a nucleic acid sequence encoding the PDE
5 under stringent conditions, and wherein,

at least one nucleotide of the oligonucleotide is 2-amino-2'-deoxyadenosine (DAP); and

the internucleoside linkages of the oligonucleotide comprises at least three alternating segments, each segment consisting of either at least
10 one phosphorothioate or at least one phosphodiester bond.

In a further aspect, there is provided a pharmaceutical composition comprising one oligonucleotide described herein and a pharmaceutically acceptable carrier.

In a further aspect, there is provided the pharmaceutical composition
15 described herein for the treatment of inflammation.

In a further aspect, there is provided the pharmaceutical composition described herein for treatment of respiratory disease.

In a further aspect, there is provided a method of treating respiratory disease in a subject comprising administering the pharmaceutical composition
20 described herein.

In a further aspect, there is provided the use of the pharmaceutical composition described herein in the preparation of a medicament for the treatment of respiratory disease.

In a further aspect, there is provided the use of the pharmaceutical
25 composition described herein for the treatment of respiratory disease.

In a further aspect, there is provided an oligonucleotide comprising the base sequence of any one of SEQ ID NOs. 1-72.

In a further aspect, there is provided an oligonucleotide consisting of any one of SEQ ID NOs. 1-127.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the efficacy of AON sequences at reducing the PDE7A mRNA expression. Flp-In T-Rex 293 cells were transfected for 24 h using 236 nM of AON sequences TOP1766 (SEQ. ID No. 30) to TOP1777 (SEQ. ID No. 41) and efficacy compared to that of a known PDE7A AON TOP1731 (SEQ. ID No. 1). TOP1731s (SEQ. ID No. 128) was used as negative control. PDE7A mRNA expression (mean $\pm$ SEM) was quantified using the Quantigene 2.0 assay, with normalization to the expression levels of the control gene β 2M. The average percentage inhibition of PDE7A mRNA compared to control non-transfected cells (Ctrl NT, dotted line) was indicated for AON treatments when greater than 20%. N=6 for Ctrl NT and n=3 for transfected samples.

Figure 2 illustrates the efficacy of AON sequences at reducing the PDE4D and PDE4B mRNA expression. Flp-In T-Rex 293 cells were transfected for 24 h using 236 nM of AON sequence against TOP1572 (SEQ. ID No. 42). TOP1572s (SEQ. ID No. 131) was used as negative control. (A) PDE4D and (B) PDE4B mRNA expression (mean $\pm$ SEM) was quantified using the Quantigene 2.0 assay, with normalization to the expression levels of the control gene β 2M. The average percentage inhibition of PDE4D and PDE4B mRNA compared to gene levels in control non-transfected cells (Ctrl NT, dotted line) was indicated for AON treatments when greater than 20%. N=6 for Ctrl NT and n=3 for transfected samples.

Figure 3 illustrates the efficacy of human AON sequences at reducing mouse PDE mRNA expression. NIH3T3 cells were transfected for 24 h with 125 nM of the human PDE7A AON sequences TOP1731 (SEQ. ID No. 1), TOP1769 (SEQ. ID No. 33), TOP1777 (SEQ. ID No. 41) and the PDE4B/4D sequence TOP1572 (SEQ ID No. 42). The efficacy of the human AON was compared to that of known mouse specific PDE7A AON sequence TOP2707-F3 (SEQ. ID No. 134) and PDE4B/4D specific sequence TOP2804-F1 (SEQ. ID No.) or control sequences TOP2707Mi2-F3 (SEQ. ID No. 125) and TOP1572s (SEQ. ID No. 131). The Quantigene 2.0 assay was used to quantify murine (A) PDE7A, (B) PDE4D or (C) PDE4B mRNA expression (mean $\pm$ SEM) normalized to β 2M levels. The average percentage inhibition of PDE mRNA compared to gene levels in non-transfected control cells (Ctrl NT, dotted line) was indicated when greater than 20% (n=3 for all tested group, except n=6 for Ctrl NT).

Figure 4 illustrates the efficacy of human AON sequences at reducing rat PDE mRNA expression. L2 cells were transfected for 24 h with 250 nM of PDE7A AON sequences TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101) or 500 nM of PDE4B/4D AON sequence TOP1572-P²M-7-DAP (SEQ ID No. 119). The efficacy of the human AON sequences was compared to that of control sequences TOP1731s-P²M-7-DAP (SEQ. ID No. 155) and TOP1572s-P²M-7-DAP (SEQ. ID No. 156). The Quantigene 2.0 assay was used to quantify rat (A) PDE7A, (B) PDE4D or (C) PDE4B mRNA expression (mean $\pm$ SEM) normalized to PPIB levels. The average percentage inhibition of PDE mRNA compared to gene levels in non-transfected control cells (Ctrl NT, dotted line) was indicated when greater than 20% (n=3 for all tested group, except n=6 for Ctrl NT).

Figure 5 illustrates the efficacy of human AON sequences at reducing monkey PDE mRNA expression. CYNOM-K1 cells were transfected for 24 h with 250 nM of PDE7A AON sequences TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No.

101), or the PDE4B/4D sequence TOP1572 (SEQ ID No. 42) or control sequences TOP1731s-P²M-7-DAP (SEQ. ID No. 155) and TOP1572s (SEQ. ID No. 131). The Quantigene 2.0 assay was used to quantify monkey (A) PDE7A, (B) PDE4D or (C) PDE4B mRNA expression (mean $\pm$ SEM)
 5 normalized to PPIB levels. The average percentage of PDE mRNA inhibition compared to gene levels in non-transfected control cells (Ctrl NT, dotted line) was indicated when greater than 20% (n=3 for all tested group, except n=6 for Ctrl NT).

Figure 6 illustrates the efficacy comparison between selected unmodified
 10 AON (PS-DNA) and DAP-containing AON sequences at reducing target mRNA expression. Flp-In T-Rex 293 cells were transfected for 24 h using 118 nM of TOP1731 (SEQ. ID No. 1), TOP1731-DAP (SEQ. ID No. 74) or 263 nM of TOP1572 (SEQ. ID. No. 42) or TOP1572-DAP (SEQ. ID No. 107). (A) PDE7A, (B) PDE4D and C) PDE4B mRNA expression (mean $\pm$ SEM) were
 15 quantified using the Quantigene 2.0 assay, with normalization to the expression levels of the control gene β 2M. The average percentage of PDE mRNA inhibition compared to gene levels in control non-transfected cells (Ctrl NT, dotted line) was indicated for AON treatments. N=6 for Ctrl NT and n=3 for transfected samples.

20 Figure 7 illustrates the efficacy comparison between selected P²M-DAP AON sequences at reducing target mRNA expression. Flp-In T-REx 293 cells were transfected for 24 h with 236 nM of PDE7A AON as PS-DNA or as various P²M or P²M-DAP versions of (A) TOP1731 (SEQ. ID No. 1), (B) TOP1769 (SEQ. ID No. 33) or (C) TOP1777 (SEQ. ID No. 41). The mRNA expression
 25 (mean $\pm$ SEM) of PDE7A was quantified using the Quantigene 2.0 assay with normalization to the expression levels of β 2M. The average percentage of PDE mRNA inhibition compared to gene level in non-transfected control (Ctrl NT, dotted line) is indicated when greater than 20%. Student t-test analyses, *p<0.05, **p<0.01, ***p<0.001. N=3 for all test groups except n=6 for Ctrl NT.

Figure 8 illustrates the efficacy comparison between selected PS-DNA and P²M-DAP-containing AON sequences at reducing target mRNA expression. Flp-In T-REx 293 cells were transfected for 24 h with 15 to 1200 nM of (A) TOP1731 (SEQ. ID No. 1), (B) TOP1769 (SEQ. ID No. 33), (C) TOP1777 (SEQ. ID No. 41) or (D, E) TOP1572 (SEQ. ID No. 42) as PS-DNA or P²M-7-DAP, respectively. Sequences TOP1731s (SEQ. ID No. 128) and TOP1777s-P²M-7-DAP (SEQ. ID No. 132) were used as controls. The Quantigene 2.0 assay was used to quantify PDE mRNA expression (mean $\pm$ SEM) relative to the expression of the control gene β 2M (n=3 for all test groups except n=6 for Ctrl NT). Non-linear fit (one phase exponential decay) curves were generated using the GraphPad Prism software. Panel F illustrates the area under the dose-response curves (AUC) and compares the percentage reduction of the AUC between P²M-7-DAP-containing AON sequences and PS-DNA.

Figure 9 illustrates the dose-response comparison between selected FANA-containing and P²M-DAP-containing AONs. Flp-In T-REx 293 cells were transfected for 24 h with 6 to 1260 nM of a combination of either FANA-containing or P²M-DAP-containing TOP1572 (SEQ. ID No. 42) and TOP1731 (SEQ. ID No. 1). (A) PDE7A, (B) PDE4D and (C) PDE4B mRNA expression (mean $\pm$ SEM) were measured using the Quantigene 2.0 assay, with normalization to the expression of the control gene β 2M. Non-linear fit (one phase exponential decay) curves were generated using the GraphPad Prism software. The percentage of PDE inhibition at the maximal concentration compared to gene levels in non-transfected cells are indicated (n=6 for Ctrl NT and n=3 for transfected conditions).

Figure 10 illustrates the comparison between selected LNA-containing and P²M-DAP-containing AONs. Flp-In T-REx 293 cells were transfected for 24 h with either 0, 238, 476 or 713 nM of a P²M-DAP-containing sequence (TOP1731-P²M-7-DAP, SEQ. ID No. 86) or LNA-containing sequence (TOP1731-LNA1, SEQ. ID No. 93). Sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132) was used as control. PDE7A mRNA expression (mean $\pm$ SEM) was measured using the Quantigene 2.0 assay, with normalization to the

expression of the control gene $\beta 2M$. N=6 for Ctrl NT and n=3 for transfected conditions.

Figure 11 illustrates the dose-response efficacy of selected PDE4B/4D and PDE7A P²M-DAP-containing AON combinations. Flp-In T-REx-293 cells
5 were transfected using 6 to 1260 nM of indicated AON combinations or control sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132). (A) PDE7A, (B) PDE4D and (C) PDE4B mRNA expressions (mean $\pm$ SEM) were measured using the Quantigene 2.0 assay and normalized to the expression of the control gene $\beta 2M$. Non-linear fit (sigmoidal dose-response/variable slope)
10 curves on transformed data (Log(X)) were generated using the GraphPad Prism software.

Figure 12 illustrates the tissue half-lives of selected PDE4B/4D and PDE7A AON in mouse lung. The amount of remaining TOP1572-P²M-7-DAP (SEQ. ID No. 119), TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP
15 (SEQ. ID No. 95) and TOP1777-P²M-7-DAP (SEQ. ID No. 101) was determined in lung tissue homogenates at different time points following administration via intra-tracheal aerosols. Each data point represents the mean $\pm$ SD of 5 mice per time point, and is expressed as the percentage of amount of AON recovered relative to time 0 h set at 100%. Non-linear
20 regression curves were generated using Graph Pad Prism and the half-life of each AON was calculated using one-phase exponential decay parameters.

Figure 13 illustrates the duration of action of selected PDE4B/4D and PDE7A P²M-DAP-containing AON combinations. Flp-In T-REx-293 cells were transfected using 378 nM of indicated AON combinations (PDE4B/4D;
25 TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101)) or control sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132). (A) PDE7A, (B) PDE4D and (C) PDE4B mRNA expressions (mean $\pm$ SEM) were measured at indicated time-points using the Quantigene
30 2.0 assay and normalized to the expression of the control gene $\beta 2M$. Data

are expressed on a log-time scale as mean $\pm$ SEM with the control non-transfected (Ctrl NT) values set at 1.0. Paired t-test was used to calculate statistical difference versus TOP1572-P²M-7-DAP+TOP1731-P²M-7-DAP (*p<0.05, **p<0.01, n=3).

- 5 Figure 14 shows the biological effect on AON on primary lung epithelial cells. Normal human bronchoepithelial (NHBE) cells were transfected for 20 h with a combination of PDE4B/4D- and PDE7A-specific P²M-DAP modified AON (250 nM total AON) (TOP1572-P²M-7-DAP (SEQ. ID No. 119) referred as TOP1572 on the "x"-axis of the figure, TOP1769-P²M-7-DAP (SEQ. ID No. 95) referred as TOP1769 on the "x"-axis of the figure) or control sequence (TOP1777s-P²M-7-DAP (SEQ. ID No. 132) referred as TOP1777s on the "x"-axis of the figure) and were stimulated with a cytomix (10 ng/mL each of TNF- α , IL-1 β and IFN- γ) for 4 h to induce gene expression and protein secretion of chemokine (MCP-1), metalloproteases (MMP-2, MMP-12) and cytokine (GM-CSF, IL1- β). Rolipram (36 μ M), a small molecule PDE4 inhibitor, was used as control. (A-G) Real-time PCR was used to quantify mRNA expression (mean $\pm$ SEM) which was normalized to the expression of a control gene (β 2M). N=6 for Ctrl NT and n=3 for transfected conditions. The percentage of inhibition compared to stimulated non-transfected cells (Ctrl NT, dotted line) are indicated. Ctrl NS represents non-transfected and non-stimulated cells. Student t-test was used to calculate statistical difference versus TOP1777s-P²M-7-DAP (*p<0.05, **p<0.01, ***p<0.001, n=3).

- Figure 15 shows the biological effect on AON on lung epithelial cells. A549 cells were transfected for 20 h with a combination of PDE4B/4D- and PDE7A-specific P²M-DAP modified AON (236 nM total AON) (PDE4B/4D; TOP1572 (SEQ. ID No. 42), TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP1731 (SEQ. ID No. 1), TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95) or TOP1777-P²M-7-DAP (SEQ. ID No. 101)) and were stimulated with IL-1 β for 6 h to induce protein secretion of chemokines (IL-8 and MCP-1). Rolipram (36 μ M), a small molecule PDE4 inhibitor, was used as control. Cell supernatants were harvested and levels

(mean $\pm$ SEM) of (A) IL-8 and (B) MCP-1 determined using ELISA. N=6 for Ctrl NT and n=3 for transfected conditions. The percentage of inhibition compared to stimulated non-transfected cells (Ctrl NT, dotted line) are indicated. Ctrl NS represents non-transfected and non-stimulated cells.

- 5 Statistical significance is shown as ** ($p < 0.01$). One-way ANOVA followed by Dunnett's post-test vs. stimulated cells (Ctrl NT).

Figure 16 illustrates a comparison of the effects of P²M-DAP modified AONs to non-P²M-DAP modified AONs (PS-DNA) on the immune response in vitro. (A) Mouse macrophage cells (RAW 264.7) or (B) human PBMC were exposed
10 to different concentrations of AONs (TOP1731 (SEQ. ID No. 1) or TOP1731-P²M-7-DAP (SEQ. ID No. 86)). Mouse macrophage cells (RAW 264.7) were also exposed to AON sequences TOP062 (SEQ. ID No.) or TOP062-P²M-11-DAP (SEQ. ID No.), TOP062-P²M-12-DAP (SEQ. ID No.), TOP057-P²M-4-DAP (SEQ. ID No.) and TOP057-P²M-5-DAP (SEQ. ID No.) against β -chain
15 (C) or AONs TOP030 (SEQ. ID No.) or TOP030-P²M-15-DAP (SEQ. ID No.), TOP031-P²M-1-DAP (SEQ. ID No.) and TOP031-P²M-5-DAP (SEQ. ID No.) against CCR3 (D). A known immunostimulatory sequence CpG (SEQ. ID No. 133) was used as positive control and levels of TNF- α and IFN- α in culture supernatants measured 24 h later (n=3-5).

20 Figure 17 illustrates a comparison of the effects of P²M-DAP modified AONs to non-P²M modified AONs (PS-DAP or PS-DNA) on the inflammatory response in the lung of rodents (mice or rat) following chronic dosing. (A) Rats were treated with selected AONs (PS-DAP; TOP004 (SEQ. ID No. 157), TOP005 (SEQ. ID No. 158), PS-DNA; TOP006 (SEQ. ID No. 159), TOP007
25 (SEQ. ID No. 160)) at the indicated dose for 14 consecutive days and the inflammatory response assessed by lung tissue histopathology. Mice were treated with selected AONs (TOP1731 (SEQ. ID No. 1) or TOP1731-P²M-7-DAP (SEQ. ID No. 86)) at indicated doses for 10 days and inflammatory response assessed in (B) bronchoalveolar lavage (BAL) and by (C) lung
30 tissue histopathology.

Figure 18 shows the effect of specific inhibition by P²M-DAP-modified PDE4B/4D and PDE7A AON on LPS-induced acute lung inflammation in mice. Inhibition of the cellular influx in the lung of LPS-exposed mice pre-treated with selected mouse (A) or human (B) AON combinations (PDE4B/4D; 5 TOP2804-P²M-7-DAP (SEQ. ID No. 138), TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP2707-P²M-7-DAP (SEQ. ID No. 136), TOP1777-P²M-7-DAP (SEQ. ID No. 101)) or control sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132) or (C) roflumilast as positive control. Differential cell counts of BAL were performed 3 h after LPS exposure and percentage inhibition was 10 determined by comparing with LPS-challenged vehicle treated animals. Values shown represent mean $\pm$ SEM (n = 5-10), Student t-test was used to calculate statistical difference versus LPS-challenged vehicle treated animals (*p<0.05, **p<0.01).

Figure 19 shows the efficacy of treatment with combined PDE4B/4D and 7A 15 P²M-DAP-modified AONs against cigarette smoke-induced lung inflammation. Inhibition of the cellular influx in the lung of smoke-exposed mice pre-treated with selected mouse (A and D) or human (B and E) AON combinations (PDE4B/4D; TOP2804-P²M-7-DAP (SEQ. ID No. 138), TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP2707-P²M-7-DAP (SEQ. ID No. 136), 20 TOP1777-P²M-7-DAP (SEQ. ID No. 101)) or control sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132) or (C and F) roflumilast as positive control. Differential cell counts of BAL were performed 16-20 h after smoke exposure and percentage inhibition was determined by comparing with smoke-exposed vehicle treated animals. Values shown represent mean $\pm$ SEM.

25 Figure 20 illustrates the efficacy comparison between selected DAP-containing and P²M-DAP-containing AON sequences at reducing target mRNA and protein expression. (A and B) TF-1 cells were transfected for 24 h with 1.34 μ M of a combination of either TOP004 (SEQ. ID No. 157) and TOP005 (SEQ. ID No. 158), or TOP062-P²M-12-DAP (SEQ. ID No. 185) and 30 TOP030-P²M-15-DAP (SEQ. ID No. 170), or TOP062-P²M-12-DAP (SEQ. ID

No. 185) and TOP031-P²M-1-DAP (SEQ. ID No. 172). The Quantigene 2.0 assay was used to quantify (A) CCR3 and (B) β -chain mRNA expression and represented as the % inhibition compared to the expression of the control untransfected cells. (C and D) TF-1 cells were transfected for 24 h with 334 or
5 668 nM total of a combination of either TOP004 (SEQ. ID No. 157) and TOP005 (SEQ. ID No. 158), or TOP062-P²M-12-DAP (SEQ. ID No. 185) and TOP030-P²M-15-DAP (SEQ. ID No. 170), or TOP062-P²M-12-DAP (SEQ. ID No. 185) and TOP031-P²M-1-DAP (SEQ. ID No. 172). Protein expression levels were measured by flow cytometry 24h after transfection. Results are
10 expressed as the mean fluorescence intensity (MFI) $\pm$ SEM (n=3). Percentage inhibition compared to control non-transfected (Ctrl NT) cells indicated above each bar.

BRIEF DESCRIPTION OF THE TABLES

15 Table 1 identifies AON sequences with specificity for the human phosphodiesterase (PDE) isoform 7A in accordance with the present invention.

Table 2 identifies AON sequences with specificity for the human phosphodiesterase (PDE) isoforms 4B and 4D in accordance with the present
20 invention.

Table 3 illustrates the homology between human AON sequences and the sequences from different animal species (mouse, rat and monkey).

Table 4 identifies the AON sequences with specificity for the human phosphodiesterase (PDE) isoforms 4B, 4D and 7A containing modified
25 chemistry in accordance with the present invention.

Table 5 identifies the oligonucleotide sequences used as control in accordance with the present invention.

Table 6 identifies AON sequences with specificity for the mouse phosphodiesterase (PDE) isoforms in accordance with the present invention.

- 5 Table 7 describes the real-time PCR primers used to quantify target genes and inflammatory markers in *in vitro* and *in vivo* models.

Table 8 describes the influx of inflammatory cells in bronchoalveolar lavages and the histopathological changes following administration of the AON sequences to the lungs of mice.

- 10 Table 9 identifies AON sequences used in *in vivo* toxicology studies in accordance with the present invention.

Table 10 identifies the AON sequences with specificity for the human β -chain and CCR3 containing modified chemistry in accordance with the present invention.

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DETAILED DESCRIPTION

- There is described herein oligonucleotides directed against phosphodiesterase (PDE) isoforms in order to downregulate the expression thereof. The oligonucleotides have a mixed phosphodiester/phosphorothioate backbone (P²M) and at least one 2-amino-2'-deoxyadenosine (DAP) nucleotide. It has been surprisingly found that the P²M and DAP modifications exhibit synergy in improving anti-PDE oligonucleotides by increasing their potency, efficacy or stability and/or decreasing their toxicity. For example, the oligonucleotides described herein preferably exhibit unexpectedly higher efficacy/toxicity ratios.
- 20
- 25

Accordingly, in an aspect, there is provided an oligonucleotide directed against a target Phosphodiesterase (PDE), wherein the oligonucleotide is capable of hybridizing to at least a portion of a nucleic acid sequence encoding the PDE under stringent conditions, and wherein,

- 5 at least one nucleotide of the oligonucleotide is 2-amino-2'-deoxyadenosine (DAP); and

the internucleoside linkages of the oligonucleotide comprises at least three alternating segments, each segment consisting of either at least one phosphorothioate or at least one phosphodiester bond.

- 10 Preferably, the target PDE is selected from the group consisting of PDE3A, PDE3B, PDE4A, PDE4B, PDE4C, PDE4D, PDE7A1, PDE7A2, PDE7A3 and PDE7B, preferably PDE7A, PDE4B and PDE4D.

- In some embodiments, ratio of phosphorothioate to phosphodiester bonds is between 30:70 and 70:30, preferably 40:60 and 60:40, 45:55 and 55:45 and
15 50:50.

In some embodiments, the oligonucleotide is at least 80% complementary to the target PDE, preferably 100%.

The oligonucleotide preferably exhibits a efficacy/toxicity ratio greater than 0.25.

- 20 In some embodiments, the oligonucleotide is between 15-25 nucleotides in length, preferably between 18-22 nucleotides in length.

In one embodiment, the oligonucleotide has a base sequence selected from the group consisting of SEQ ID NOs. 1-72, wherein at least one adenosine is replaced with DAP, and is preferably SEQ ID NOs. 1, 33, 36, 41 and 42.

In a preferably embodiment, the oligonucleotide comprises, preferably consists of, any one of SEQ ID NOs. 81, 86, 90, 92, 95, 97, 99, 101, 103, 105, 114, 119, 121, 123, 125 and 127.

5 In a further aspect, there is provided a pharmaceutical composition comprising one oligonucleotide described herein and a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical compositions comprises at least two of the oligonucleotides described herein, preferably directed against PDE7A and PDE4B, or PDE7A and PDE4D respectively. In a preferably embodiment, the oligonucleotide directed against PDE4B or
10 PDE4D also downregulates the other.

In a further aspect, there is provided the pharmaceutical composition described herein for the treatment of inflammation.

In a further aspect, there is provided the pharmaceutical composition described herein for treatment of respiratory disease, preferably at least one
15 of chronic obstructive pulmonary disease, asthma, eosinophilic cough, bronchitis, acute and chronic rejection of lung allograft, sarcoidosis, pulmonary fibrosis, rhinitis, sinusitis, viral infection or a neoplastic disease.

In a further aspect, there is provided a method of treating respiratory disease in a subject comprising administering the pharmaceutical composition
20 described herein.

In a further aspect, there is provided the use of the pharmaceutical composition described herein in the preparation of a medicament for the treatment of respiratory disease.

In a further aspect, there is provided the use of the pharmaceutical
25 composition described herein for the treatment of respiratory disease.

In a further aspect, there is provided an oligonucleotide comprising the base sequence of any one of SEQ ID NOs. 1-72, preferably wherein at least one adenosine is replaced with DAP.

In a further aspect, there is provided an oligonucleotide consisting of any one
5 of SEQ ID NOs. 1-127.

The terms "nucleic acid" and "nucleic acid molecule" as used interchangeably herein, refer to a molecule comprised of nucleotides, i.e., ribonucleotides, deoxyribonucleotides, or both. The term includes monomers and polymers of ribonucleotides and deoxyribonucleotides, with the ribonucleotide and/or
10 deoxyribonucleotides being connected together, in the case of the polymers, via 5' to 3' linkages. However, linkages may include any of the linkages known in the nucleic acid synthesis art including, for example, nucleic acids comprising 5' to 2' linkages. The nucleotides used in the nucleic acid molecule may be naturally occurring or may be synthetically produced analogues that
15 are capable of forming base-pair relationships with naturally occurring base pairs.

"Bases" includes any one of the natively found purine and pyrimidine bases, adenine (A), thymine (T), cytosine (C), guanine (G) and uracil (U), but also any modified or analogous forms thereof. Examples of non-naturally occurring
20 bases that are capable of forming base-pairing relationships include, but are not limited to, aza and deaza pyrimidine analogues, aza and deaza purine analogues, and other heterocyclic base analogues, wherein one or more of the ring atoms and/or functional groups of the purine and pyrimidine rings have been substituted by heteroatoms, e.g., carbon, fluorine, nitrogen,
25 oxygen, sulfur, and the like. Preferably, such bases include, but are not limited to, inosine, 5-methylcytosine, 2-thiothymine, 4-thiothymine, 7-deazaadenine, 9-deazaadenine, 3-deazaadenine, 7-deazaguanine, 9-deazaguanine, 6-thioguanine, isoguanine, 2,6-diaminopurine, hypoxanthine, and 6-thiohypoxanthine. Bases may also include, but are not limited to, 5-
30 fluorocytosine, 5-bromocytosine, 5-iodocytosine, isocytosine, N4-

methylcytosine, 5-iodouracil, 5-fluorouracil, 4-thiouracil, 2-thiouracil, (E)-5-(2-bromovinyl)uracil, N6-methyladenine, 2-chloroadenine, 2-fluoroadenine, 2-chloroadenine, N6-cyclopropyl-2,6-diaminopurine, nicotinamide, 2-aminopurine, 1,2,4-triazole-3-carboxamide.

- 5 The term "nucleic acid backbone" or "internucleoside linkage" as used herein refers to the structure of the chemical moiety linking nucleotides in a molecule. This may include structures formed from any and all means of chemically linking nucleotides.

- The term "oligonucleotide" as used herein refers to a nucleic acid molecule
10 from about 2 to about 100 nucleotides, and in increasing preferability, 2 to 80 nucleotides, 4 to 35 nucleotides, 10 to 30 nucleotides, 15 to 25 nucleotides, and 18 to 22 nucleotides.

- Moreover, the skilled artisan recognizes that substantially similar nucleic acid sequences encompassed by this invention are also defined by their ability to
15 hybridize, under stringent conditions (for example, 0.5 x SSC, 0.1% SDS, 60°C) with the sequences exemplified herein, or to any portion of the nucleotide sequences disclosed herein and which are functionally equivalent to any of the nucleic acid sequences disclosed herein. Stringency conditions can be adjusted to screen for moderately similar fragments, such as
20 homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions involves a series of washes starting with 6 x SSC, 0.5% SDS at room temperature for 15 min, then
25 repeated with 2 x SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2 x SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of highly stringent conditions involves the use of higher temperatures in which the washes are identical to those above except the temperature of the final two 30 min. washes in 0.2 x SSC, 0.5% SDS was increased to 60°C. Another

preferred set of very highly stringent conditions involves the use of two final washes in 0.1 x SSC, 0.1% SDS at 65°C.

The terms "treatment", "treating", "therapy" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect.

- 5 The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or amelioration of an adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a subject as previously defined, particularly a human, and
- 10 includes:
- (a) preventing a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it;
 - (b) inhibiting a disease, i.e., arresting its development; or
 - 15 (c) relieving a disease, i.e., causing regression of the disease.

The term "pharmaceutically acceptable" as it is used herein with respect to carriers, excipients, surfactants and compositions refers to substances which are acceptable for use in the treatment of a subject patient that are not toxic or otherwise unacceptable for administration by any of the routes herein

20 described.

The formulations of the present invention are preferably administered directly to the site of action and, thus, preferably are topical, including but not limited to, oral, intrabuccal, intrapulmonary, rectal, intrauterine, intratumor, nasal, intrathecal, inhalable, transdermal, intradermal, intracavitary, iontophoretic,

25 ocular, vaginal, intraarticular, otical, transmucosal, rectal, slow release or enteric coating formulations. Without limiting any of the foregoing, formulations of the present invention may also be intracranial, intramuscular, subcutaneous, intravascular, intraglandular, intraorgan, intralymphatic,

intraperitoneal, intravenous, and implantable. The carriers used in the formulations may be, for example, solid and/or liquid carriers.

Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Pa., 1985, for other carriers that would be
5 suitable for combination with the present oligonucleotide compounds to render compositions/formulations suitable for administration to treat respiratory disease.

Optionally, the presently described oligonucleotides may be formulated with a variety of physiological carrier molecules. The presently described
10 oligonucleotides may also be complexed with molecules that enhance their ability to enter the target cells. Examples of such molecules include, but are not limited to, carbohydrates, polyamines, amino acids, peptides, lipids, and molecules vital to cell growth. For example, the oligonucleotides may be combined with a lipid, the resulting oligonucleotide/lipid emulsion, or liposomal
15 suspension may, inter alia, effectively increase the in vivo half-life of the oligonucleotide.

The pharmaceutical compositions provided herein may comprise oligonucleotide compounds described above and one or more pharmaceutically acceptable surfactants. Suitable surfactants or surfactant
20 components for enhancing the uptake of the oligonucleotides of the invention have been previously described in U.S. Application Publication No. 2003/0087845, the contents of which are incorporated herein with respect to surfactants. The application states that suitable surfactants "...include synthetic and natural as well as full and truncated forms of surfactant protein
25 A, surfactant protein B, surfactant protein C, surfactant protein D and surfactant protein E, di-saturated phosphatidylcholine (other than dipalmitoyl), dipalmitoylphosphatidylcholine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine; phosphatidic acid, ubiquinones, lysophosphatidylethanolamine,
30 lysophosphatidylcholine, palmitoyl-lysophosphatidylcholine,

dehydroepiandrosterone, dolichols, sulfatidic acid, glycerol-3-phosphate, dihydroxyacetone phosphate, glycerol, glycerol-3-phosphocholine, dihydroxyacetone, palmitate, cytidine diphosphate (CDP) diacylglycerol, CDP choline, choline, choline phosphate; as well as natural and artificial lamellar
5 bodies which are the natural carrier vehicles for the components of surfactant, omega-3 fatty acids, polyenic acid, polyenoic acid, lecithin, palmitinic acid, non-ionic block copolymers of ethylene or propylene oxides, polyoxypropylene, monomeric and polymeric, polyoxyethylene, monomeric and polymeric, poly (vinyl amine) with dextran and/or alkanoyl side chains, Brij
10 35TM, Triton X-100TM and synthetic surfactants ALEC TM, Exosurf TM, Survan TM and Atovaquone TM, among others. These surfactants may be used either as single or part of a multiple component surfactant in a formulation, or as covalently bound additions to the 5' and/or 3' ends of the AONs.

15 The oligonucleotide component of the present compositions may be contained in a pharmaceutical formulation within a lipid particle or vesicle, such as a liposome or microcrystal. As described in U.S. Patent No. 6,025,339, the lipid particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the oligonucleotide is contained therein. Positively charged lipids
20 such as N-[1-(2, 3-dioleoyloxy) propyl]-N, N, N-trimethylammoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Pat. Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.;
25 4,921,757 to Wheatley et al.; etc.

The composition of the invention may be administered by any means that transports the oligonucleotide compound to the desired site, such as for example, the lung. The oligonucleotide compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are
30 preferably administered by inhalation of an aerosol comprised of respirable particles that comprise the oligonucleotide compound.

The oligonucleotides may be formulated to be administered in a dry powder inhaler, metered dose inhaler, nebulizer, soft mist inhaler and by any other suitable device having the capacity to deliver oligonucleotides to the lungs via inhalation route.

- 5 The composition of the present invention may be administered into the respiratory system as a formulation including particles of respirable size, e.g. particles of a size sufficiently small to pass through the nose, mouth and larynx upon inhalation and through the bronchi and alveoli of the lungs. In general, respirable particles range from about 0.5 to 10 microns in size.
- 10 Particles of non-respirable size that are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably thus minimized. For nasal administration, a particle size in the range of 10-500 μm is preferred to ensure retention in the nasal cavity.
- 15 A solid particulate composition comprising the oligonucleotide compound may optionally contain a dispersant that serves to facilitate the formation of an aerosol as well as other therapeutic compounds. A suitable dispersant is lactose, which may be blended with the antisense compound in any suitable ratio, e.g., a 1 to 1 ratio by weight.
- 20 Liquid pharmaceutical compositions of active compound (the oligonucleotide compound(s)) for producing an aerosol may be prepared by combining the oligonucleotide compound with a suitable vehicle, such as sterile pyrogen free water or phosphate buffered saline.

- The aerosols of liquid particles comprising the oligonucleotide compound may
- 25 be produced by any suitable means, such as with a nebulizer. Nebulizers are commercially available devices that transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for
 - 30 use in nebulizers comprise the active oligonucleotide ingredient in a liquid

carrier in an amount of up to 40% w/w preferably less than 20% w/w of the formulation. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the
5 formulation is not prepared sterile, for example, methyl hydroxybenzoate, anti-oxidants, anti-bacterials, flavorings, volatile oils, buffering agents and emulsifiers and other formulation surfactants.

The aerosols of solid particles comprising the active oligonucleotide compound(s) and a pharmaceutically acceptable surfactant may likewise be
10 produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles that are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. The active oligonucleotide
15 ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation
20 through a valve adapted to deliver a metered volume, typically from 10 to 150 μ L, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or hydrofluoroalkanes and mixtures thereof. The formulation may additionally
25 contain one or more co-solvents, for example, ethanol, emulsifiers and other formulation surfactants, such as oleic acid or sorbitan trioleate, anti-oxidants and suitable flavoring agents.

The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator at a rate of from about 1 to 150 litres per minute.

The present invention will be more readily understood by referring to the examples that are given to illustrate the following invention rather than to limit its scope.

5

EXAMPLES

METHODS

Cell culture

A549 cells (human lung carcinoma; ATCC; #CCL-185) were grown in Ham's F12 media containing 10% FBS, 100 U/mL Penicillin and 100 μ g/mL Streptomycin. Flp-In T-Rex 293 cells (human transformed embryonic kidney; Invitrogen; #R780-07) were cultivated in DMEM containing 10% FBS, penicillin 100 U/mL and streptomycin 100 μ g/mL. NHBE primary cells (normal human bronchial epithelial; Cederlane; #CC-2540) were cultivated in BEBM media (500 mL) supplemented with 2 mL BPE, 0.5 mL Hydrocortisone, 0.5 mL hEGF, 0.5 mL Epinephrine, 0.5 mL Transferrin, 0.5 mL Insulin, 0.5 mL Retinoic Acid, 0.5 mL Triiodothyronine (Cederlane, cat# CC-3170). CYNOM-K1 cells (cynomolgus monkey skin embryonic; ECACC; #90071809) were cultivated in EMEM (EBSS) media containing 10% FBS, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/mL Penicillin and 100 μ g/mL Streptomycin. L2 cells (rat lung epithelial; ATCC; #CRL-149) were cultivated in F12K media containing 10% FBS, penicillin 100 U/mL and streptomycin 100 μ g/mL. NIH3T3 cells (mouse fibroblast; ATCC; #CRL-1658) were cultivated in DMEM media containing 10% BCS, penicillin 100 U/mL and streptomycin 100 μ g/mL. RAW264.7 cells (Leukaemic monocyte macrophage; ATCC; # TIB-71) were cultivated in DMEM media containing 10% FBS, 1mM sodium pyruvate, penicillin 100 U/mL and streptomycin 100 μ g/mL. Human peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers. PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of EDTA K3 blood from normal donors. PBMC were

plated at 0.2×10^6 cells/200 μ L in 48-well plates (for suspension cells) in AIM-V culture media. TF-1 cells (erythroleukemia) were maintained in RPMI-1640 supplemented with 2 mM L-glutamine, 10 mM HEPES, 4.5g/L glucose, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, 10% non-inactivated FBS and 2
5 ng/mL rhGM-CSF.

Preparation of antisense oligonucleotides (AON)

Phosphorothioate-DNA (PS-DNA) AON (Sigma Genosys), Phosphorothioate-FANA (PS-FANA) AON (Topigen, Montreal or UCDNA, Calgary), DAP, P²M
10 and P²M-DAP AON (Biospring, Germany), 2'-OMe AON (Biospring, Germany) or Lock nucleic acid (LNA) AON (Exiqon, USA) were resuspended in sterile water and diluted in Opti-MEM for *in vitro* transfection or diluted in PBS for *in vivo* studies.

AON transfection of cells

15 A549 cells were seeded at 0.25×10^6 cells/mL (200 μ L per well in 48-well plates) in HAM-F12 medium supplemented with 10% serum without antibiotics then incubated overnight at 37°C prior to transfection with AON/Lipofectamine complexes (ratio of 1 μ g AON: 1 μ L Lipofectamine 2000). Flp-In T-Rex 293
cells were seeded at 0.9×10^6 cells/mL (200 μ L per well in 48-well plates) in
20 DMEM medium supplemented with 10% serum without antibiotics then incubated overnight at 37°C prior to transfection as described above. NHBE cells were seeded at 0.5×10^6 cells/mL (200 μ L per well in 48-well plates) in BEBM complete medium without antibiotics for 24 h prior to transfection as described. L2 cells were seeded at 1.75×10^6 cells/mL (400 μ L per well in
25 24-well plates) in F12K medium supplemented with 10% serum without antibiotics then incubated overnight at 37°C and transfected as described above. CYNOM-K1 cells were seeded at 0.25×10^6 cells/mL (200 μ L per well in 48-well plates) in EMEM medium supplemented with 10% serum without
antibiotics then incubated overnight at 37°C prior to transfection with
30 AON/Lipofectamine complexes (ratio of 1 μ g AON: 2 μ L Lipofectamine 2000).

NIH 3T3 cells were seeded at 0.25×10^6 cells/mL (200 μ L per well in 48-well plates) in DMEM medium supplemented with 10% serum without antibiotics then incubated overnight at 37°C prior to transfection with AON/Lipofectamine complexes (ratio of 1 μ g AON: 3 μ L Lipofectamine 2000). The day before the
5 transfections, TF-1 cells were diluted as to reach a density between 0.6 and 0.8×10^6 cells/mL the following day. The day of transfection, cells were plated in 12 well-plates at a density of 0.5×10^6 cells/well in 400 μ L growth media without antibiotics and then transfected with AONs mixed with the Lipofectamine 2000 transfection reagent according to a ratio of 1 μ g nucleic
10 acid: 1 μ l lipid. The complexes were incubated for 20 min. and added to the cells for 24h treatment at 37°C, 5% CO₂.

RNA extraction

RNA was extracted from cell pellets according to the RNeasy mini kit protocol using the QiaVac 24 manifold from Qiagen and treated with DNase
15 according to Qiagen's procedure. RNA was quantified using the RiboGreen reagent according to the manufacturer's protocol (Invitrogen).

Reverse transcription (RT)

Preparation of first-strand cDNA was performed using the Superscript First-Strand Synthesis System for RT-PCR kit (Invitrogen), in a total reaction
20 volume of 20 μ L. 1 μ g of RNA was first denatured at 65°C for 5 min., with 0.5 mM of each dNTPs, 0.5 μ g of oligo (dT)₁₈, 2 pmol of PDE4B gene specific reverse primer (5'- tctttgtctccctgctgga-3') and chilled on ice for at least 1 min. The mixture was incubated at 42°C for 2 min. and a second pre-mix containing 1X First-Strand Buffer, 10 mM DTT, and 40 units of SuperScript II
25 RT was added. Reactions were incubated at 42°C for 10 min., at 50°C for 1 h and inactivated by heating at 70°C for 15 min.

Real-time PCR

PCR reaction mixtures were performed with 3 μ L of cDNA reaction in a total volume of 20 μ L in presence of 0.4 mM of each PCR primer and 4 μ L of LC FastStart DNA Master SYBR Green 1 PLUS. Step 1 (Denaturation): 95°C, 10 min (slope 20°C/sec); Step 2 (Cycles x 40): 95°C, (slope 20°C/sec); 57°C or 59°C, 5 sec. (slope 20°C/sec) 72°C, 10 sec. (slope 20°C/sec); Step 3 (Melting curve): 95°C, (slope 20°C/sec); 70°C, 30 sec. (slope 20°C/sec); 95°C, 0 sec. (slope 0,1°C/sec); Step 4 (Cool): 40°C, 30 sec (slope 20°C/sec)

PCR primer sequences used for each gene are described in Table 6. Quantification of PCR products was performed using the RelQuant program (Roche).

Quantigene assay

Cells were harvested and lysed for 30 min at 55°C in 1X lysis mixture from the Quantigene® 2.0 kit (Affymetrix). Cell lysates were hybridized overnight at 55°C in Quantigene® 2.0 capture plates in the presence of specific probe sets and mRNA signal was amplified (at 55°C) and detected (at 50°C). mRNA expression was linearly quantified by luminescence signal using the Luminoskan.

Protein quantification

For β -chain or CCR3 protein measurements, TF-1 were harvested 24h post-transfection, washed twice with PBS, and incubated with eotaxin-biotin or IL-3-biotin for 1 h at 4°C. Avidin-FITC was then added and cells incubated for 30 min at 4°C. Cells were washed twice with PBS and fixed in 4% paraformaldehyde before flow cytometry analysis.

25

In vitro functional assays

NHBE cells were stimulated for 4 h with 10 ng/mL cytomix (TNF- α + IL-1 β + IFN- γ) at the end of the transfection period. A549 cells were stimulated for 6 h with 10 ng/mL IL-1 β at the end of the transfection period. The mRNA
5 expression of inflammatory markers was analyzed by real-time PCR. The protein secretion of was measured in cells supernatant by multiplex ELISA (SearchLight service, Aushon), with normalization to the cell viability as measured by alamarBlue assay.

In vitro immunostimulation assays

10 RAW264.7 cells were seeded at 0.13×10^6 cells/mL in complete media without antibiotics in 24-well plates and incubated in the presence of AON sequences or control CpG immunostimulatory sequences for 24 h. PBMC were seeded at 1×10^6 cells/mL with AIM-V media without antibiotics in 48-well plates and treated as described above. Following treatment, culture
15 supernatants were collected and protein content assayed by ELISA following the manufacturer's directions.

Half-life determination in mouse lung

CD-1 male mice (25-30 g, Charles River) were acclimated to the animal facility (Mispro, Montreal, Canada) for approximately one week prior to the
20 beginning of treatment. Mice were anesthetized with isoflurane (or ketamine/xylazine for t=0 h time point) and intubated. Aerosols of AONs (0.2 mg/kg) were administered into the trachea using a microsyrayer (Penn-Century). Immediately after AON delivery (time = 0 h) or at various time-points following delivery (time = 2 h, 8 h, 16 h, 24 h, 48 h, 72 h and 144 h),
25 mice were sacrificed using ketamine/xylazine. The lungs were removed, snap frozen in liquid nitrogen and stored at -80°C until preparation of tissue homogenates. Lung tissues were homogenized in lysis buffer, centrifuged, and the resulting supernatants were snap frozen. A Hybridation-Enzyme-

Linked-Immunosorbent-Assay (H-ELISA) method was used to perform quantification of each AON using specific probe sets. Standard curves were prepared for each AON using mouse lung tissue homogenate as a matrix.

Rodent model of lung inflammation

- 5 For all the animal studies the housing and care of mice (male C57Bl/6, Charles Rivers Laboratory) used in this study were provided according to protocols approved by Mispro Biotech's Institutional Animal Care and Use Committee, in conformity with Canadian Council on Animal Care (CCAC) guidelines. On days 1 to 5 and 8 to 12, groups of mice were anesthetized
10 with isoflurane and intubated. Aerosols of vehicle (endotoxin-free PBS) or AONs or administered into the trachea using a microsprayer (Penn-Century). Bronchoalveolar lavages (BAL) were performed 24 h after the last treatment and differential cell counts were determined on at least 300 cells using standard morphological criteria. Lung tissues were harvested, formalin fixed
15 and paraffin sections prepared. Histopathological evaluations were performed on Hematoxylin & Eosin stained slides.

The rat studies were performed at ITR Laboratories Canada (Baie d'Urfe, QC) in compliance with GLP regulations. Briefly, Male and female Sprague-Dawley rats received 14 consecutive doses of vehicle or 5 mg/kg of mg/kg of
20 TOP004/TOP005 or TOP006/TOP007 (in a 1:1 w/w ratio in saline) administered daily as aerosols using a inhalation exposure system. The animals were examined 1-2 times daily for clinical symptoms including a qualitative assessment of food consumption, and body weight was measured weekly. Electrocardiographic (ECG) activity was recorded and ophthalmic
25 examinations were conducted for animals pre-study and on Day 14.

One day after the last dose (Day 15), the rats (8/sex/group) were euthanized. Terminal procedures included complete gross necropsy examination, collection and preservation of approximately 40 tissues, and measurement of the weights of all major organs. Respiratory tract tissues (nasal cavity,

nasopharynx, larynx, pharynx, trachea, bronchi, lungs including carina and bronchial lymph nodes) from all animals were examined by light microscopy, and all collected tissues was examined for all high dose and control group animals.

5 **Mouse model of Lipopolysaccharide (LPS)-induced lung inflammation**

On day 1, groups of mice were anesthetized with isoflurane and intubated. Aerosols of vehicle (endotoxin-free PBS), AONs or controls were administered into the trachea using a microsprayer (Penn-Century). On day 1, other groups of mice were treated by gavage with vehicle (0.5% methylcellulose) or roflumilast (Rasayan). One hour after drug treatment, mice were exposed to saline or LPS (0.04 mg/mL) for 15 min in a plexiglass chamber. BAL were performed 3 h post-challenge and differential cell counts were determined on at least 300 cells using standard morphological criteria.

Mouse model of cigarette smoke-induced lung inflammation

On days 1, 3, 6 and 8, or just 6 and 8 groups of mice were anesthetized with ketamine and xylazine (60 mg/kg and 12 mg/kg, i.p.) and intubated. Aerosols of vehicle (endotoxin-free PBS), AONs or controls were administered into the trachea using a microsprayer (Penn-Century). On days 6 to 9, other groups of mice were treated by gavage with vehicle (0.5% methylcellulose) or roflumilast (Rasayan). On days 6 to 9 (3 h after drug treatment), mice were nose-only exposed to the smoke of two 2R4f reference cigarettes (University of Kentucky) per day. Cigarette smoke was delivered to mice using a nose-only exposure system (Proto-Werx), at a rate of 1 puff (20 ml) per min. BAL were performed 18-24 h after the last smoke exposure and differential cell counts were determined on at least 300 cells using standard morphological criteria.

**Example 1: Efficacy of AON
directed against human PDE7A**

The sequences of the AON directed against the phosphodiesterase (PDE) isoform 7A are presented in Table 1. The potency of some selected phosphorothioate (PS) AON sequences (PS-DNA) is demonstrated in Figure 1 which shows the reduction in gene expression following transfection with indicated AON sequence in the Flp-In T-Rex 293 cell line. The specificity of the selected AON sequences was assessed by comparing their efficacy at reducing PDE7A mRNA levels with the effect of a control sense sequence TOP1731s (SEQ. ID No. 128). AON comparative activity listed in Table 1 is expressed as the average percentage inhibition of PDE7A mRNA relative to untransfected controls (Ctrl NT).

**Example 2: Efficacy of AON
directed against human PDE4D and PDE4B**

The sequences of the AON directed against the PDE isoforms 4D and 4B are presented in Table 2. The potency of some selected PS-DNA AON sequences is demonstrated in Figure 2 which shows the reduction in gene expression following transfection with indicated AON sequences in the Flp-In T-Rex 293 cell line. The specificity of the selected AON sequences was assessed by comparing their efficacy at reducing PDE4D (Figure 2A) and PDE4B (Figure 2B) mRNA levels with the effect of a control sense sequence TOP1572s (SEQ. ID No. 131). AON comparative activity listed in Table 2 is expressed as the average percentage inhibition of PDE4D and PDE4B mRNA relative to untransfected controls.

**Example 3: Efficacy of human AON sequences
at reducing PDE mRNA levels across species**

Some selected AON sequences targeting the human PDE genes were designed to maximize homology with the PDE gene sequence from other

species, specifically the mouse, rat and monkey sequences. Table 3 describes the sequence homology and number of mismatch of selected AON sequences across these different species. Figure 3 shows the specific reduction in PDE7A (Fig. 3A), PDE4D (Fig. 3B) and PDE4B (Fig. 3C) mRNA levels by some selected AON (PDE7A; TOP1731 (SEQ. ID No. 1), TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769 (SEQ. ID No. 33), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777 (SEQ. ID No. 41), TOP1777-P²M-7-DAP (SEQ. ID No. 101), TOP2707-F3 (SEQ. ID No. 134); PDE4B/4D, TOP1572 (SEQ. ID No. 42), TOP1572-P²M-7-DAP (SEQ. ID No. 119), TOP2804-F1 (SEQ. ID No. 137)) following transfection for 24 h in NIH 3T3, a mouse cell line. The inhibitory activity of the selected AON sequences was also observed against the rat PDE mRNA in L2 cells (Figure 4A-C) and the monkey PDE mRNA in CYNOM-K1 cells (Figure 5A-C).

Example 4: AON with both P²M and DAP modifications demonstrated increased efficacy for target mRNA knockdown

This example relates to the enhanced efficacy of specific AONs against various mRNA targets when P²M and DAP modifications are incorporated into the chemistry of the AON. Tables 4a-4d and 10a-10b describe the compositions of AON modified with DAP residues and P²M linkages.

All adenosine bases of selected PS AON sequences were replaced with 2-amino-2'-deoxyadenosine bases (PS-DAP) and efficacy compared to unmodified PS-DNA AON sequences for their efficacy at reducing target mRNA expression in Flp-In T-Rex 293 cells transfected for 24 h. As shown in Figure 6, the DAP modification didn't provide increased efficacy of TOP1731 (PS-DNA; TOP1731 (SEQ. ID No. 1), PS-DAP; TOP1731-DAP (SEQ. ID No. 74)) at reducing PDE7A (Figure 6A) or TOP1572 (PS-DNA; TOP1572 (SEQ. ID No. 42), PS-DAP; TOP1572-DAP (SEQ. ID No. 107)) at reducing PDE4D (Figure 6B) and PDE4B (Figure 6C) compared to an unmodified version.

In Figure 7, Flp-In T-Rex 293 cells were transfected for 24 h with 236 nM of a PDE7A-specific AON (A) TOP1731 (SEQ. ID No. 1), (B) TOP1769 (SEQ. ID No. 33) or (C) TOP1777 (SEQ. ID No. 41) either in phosphorothioate composition (PS-DNA) or containing P²M linkage modifications (P²M; TOP1731-P²M-7 (SEQ. ID No. 85), TOP1731-P²M-9 (SEQ. ID No. 89), TOP1731-P²M-10 (SEQ. ID No. 91), TOP1769-P²M-7 (SEQ. ID No. 94), TOP1769-P²M-9 (SEQ. ID No. 96), TOP1769-P²M-10 (SEQ. ID No. 98), TOP1777-P²M-7 (SEQ. ID No. 100), TOP1777-P²M-9 (SEQ. ID No. 102), TOP1777-P²M-10 (SEQ. ID No. 104)) as indicated in the legend provided in Figure 7. Modification of TOP1731, TOP1769 or TOP1777 sequence with P²M linkages, (TOP1731-P²M-7 (SEQ. ID No. 85) TOP1769-P²M-7 (SEQ. ID No. 94) TOP1777-P²M-7 (SEQ. ID No. 100)) enhanced the efficacy of the AON to inhibit target gene expression, clearly showing an advantage of this modification for AON activity (Figure 7A-C).

The DAP base modification was incorporated into AON sequences containing P²M linkage modifications. Figure 7 shows that DAP base further enhanced the efficacy of P²M modified AON sequences (TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101)) to inhibit target gene expression, clearly showing a synergistic effect between the DAP modification and the P²M linkage modification for AON activity (Figure 7A-C).

In Figure 8, Flp-In T-Rex 293 cells were transfected for 24 h with increasing concentrations ranging between 15 and 1200 nM of PDE7A-specific AON (A) TOP1731 (SEQ. ID No. 1), (B) TOP1769 (SEQ. ID No. 33) or (C) TOP1777 (SEQ. ID No. 41) or PDE4B/4D-specific AON (D-E) TOP1572 (SEQ. ID No. 42) either in phosphorothioate composition (PS-DNA) or containing DAP base and P²M linkage modifications (P²M-DAP; TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101), TOP1572-P²M-7-DAP (SEQ. ID No. 119)) as indicated in the legend provided in Figure 8. Results in Figure 8 show that not only DAP base and P²M linkage modifications enhance the activity of the AON

sequences compared to PS-DNA but also show that these modifications surprisingly increase the maximal target mRNA inhibition that the AON sequence can reach (Figure 8A-F). The increased activity is surprising as a lower phosphorothioate/phosphodiester ratio is traditionally viewed to decrease stability (in fact, P²M-7-DAP indeed have a short half-life compared to PS-DNA, see Example 5 below). Furthermore, the advantageous increase in the maximal target mRNA inhibition is particularly surprising since changes in stability would likely affect activity, but not the maximal inhibition. As noted above in Example 4, modifying PS-DNA with DAP alone had little effect on inhibition.

Figures 9 and 10 compare the activity of DAP base and P²M linkage modifications to other modifications used to improve the activity of AON. In Figure 9, Flp-In T-Rex 293 cells were transfected for 24 h with increasing concentrations ranging between 6 and 1260 nM of a combination of either FANA-containing or P²M-DAP-containing versions of the PDE4B/4D AON sequence (TOP1572-P²M-7-DAP (SEQ. ID No. 119); TOP1572-F2 (SEQ. ID No. 106)) and the PDE7A AON sequence (TOP1731-P²M-7-DAP (SEQ. ID No. 86); TOP1731-F3 (SEQ. ID. No. 73)). Similarly to what was observed in the comparative analysis with unmodified PS-DNA AON sequences, results show that the P²M-DAP modifications have greater maximal target mRNA inhibition compared to FANA-containing AON sequences (Figure 9A-C).

In Figure 10, Flp-In T-Rex 293 cells were transfected for 24 h with increasing concentrations up to 713 nM of a locked nucleic acid (LNA)-containing or P²M-DAP-containing version of the PDE7A AON sequence (TOP1731-P²M-7-DAP (SEQ. ID No. 86); TOP1731-LNA1 (SEQ. ID. No. 93)). LNA AON have been shown to be very potent at inhibiting mRNA expression and have been describe as the most promising chemical modification (Kurreck J 2003, Eur. J. Biochem, 270:1628-1644; Jepsen and Wengel 2004, Curr. Opin. Drug Discov. Devel., 7:188-194). Results in Figure 10 show that the DAP base and P²M linkage modifications enhance the activity of the AON sequences compared to LNA.

In Figure 11, Flp-In T-Rex 293 cells were transfected for 24 h with increasing concentrations up to 1260 nM of combined PDE4B/4D and PDE7A P²M-7-DAP-containing AONs (PDE4B/4D: TOP1572-P²M-7-DAP (SEQ. ID No. 119); PDE7A: TOP1731-P²M-7-DAP (SEQ. ID No. 86); TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101) or the control sequence TOP1777s-P²M-7-DAP (SEQ. ID No. 132). Results in Figure 10 show that all combinations of PDE4B/4D and PDE7A AON sequences provide potent target mRNA knock down.

In Figure 20, TF-1 cells were transfected for 24 h with increasing concentrations up to 1.34 μ M of combined β -chain and CCR3 PS-DAP (TOP004 (SEQ. ID No. 157) and TOP005 (SEQ. ID No. 158)) or P²M-DAP-containing AONs (β -chain: TOP062-P²M-12-DAP (SEQ. ID No. 185); CCR3: TOP031-P²M-1-DAP (SEQ. ID No. 172); TOP030-P²M-15-DAP (SEQ. ID No. 170)). Results in Figure 20 show that all combinations of P²M-DAP-containing AON AON sequences provide more potent target mRNA and protein knock down.

Example 5: AON modified with P²M-DAP have prolonged duration of action

This example relates to the prolonged efficacy of P²M-DAP-containing AONs. P²M modifications are expected to reduce the stability of the AON due to the lower content of PS linkages, rendering it less resistant to nucleosidase digestion, which would shorten its activity in vitro and in vivo. The half-life of P²M-DAP-containing AONs was assessed in lung tissue. P²M-DAP-containing AONs were administered to mice via intra-tracheal aerosol and AON content measured at different time-points following administration. Figure 12 shows that the tissue half-life the P²M-DAP-containing AONs are shorter than 5 h. The short half-life of the P²M-DAP-containing AONs is in contrast to the half-life measured for the unmodified PS-DNA AON sequences. TOP1731 (SEQ. ID No. 1) and TOP1572 (SEQ. ID No. 42) lung tissue half-life is 22 h and 26 h, respectively. AON sequences TOP1731-DAP

(SEQ. ID No. 74) and TOP1572-DAP (SEQ. ID No. 107) with only the DAP-base modification have comparable half-life to the unmodified PS-DNA sequences.

In Figure 13, Flp-In T-Rex 293 cells were transfected for 24 h with 378 nM
5 (total AON) of a combination of PDE4B/4D- and PDE7A-specific AON and target gene expression assessed over time sequences (PDE4B/4D; TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101)) or control sequence (TOP1777s-P²M-7-DAP (SEQ.
10 ID No. 132).

Surprisingly, despite a relatively short half-life, the DAP base and P²M linkage modifications of the AON sequences have sustained inhibitory activity up to 72 h in vitro (Figure 13A-C).

Example 6: Effect AON modified with P²M-DAP chemistry
15 **on biological function of cells**

This example relates to efficacy of P²M-DAP-containing AON on the biological function of different cell types, specifically the secretion of cytokines and chemokines. Cytokines and chemokines are important mediators of cell activation and recruitment while metalloproteases (MMP) are important
20 modulators of tissue remodelling. Lung epithelial cells can play a role in the pathophysiology of inflammatory respiratory diseases through the secretion of chemokines that lead to the recruitment of immune cells, such as neutrophils and monocytes/macrophages, cytokines and metalloproteases. Interleukin-8 (IL-8/CXCL8) and monocyte chemoattractant protein-1 (MCP-1/CCL2) levels
25 are increased in COPD patients (Szilasi M *et al.* Pathology Oncology Research. 2006, 12:52-60). The levels of IL-8 and MCP-1 may be involved in neutrophil and macrophage recruitment respectively. Similarly, MMPs may contribute to the pathogenesis of COPD (Baraldo et al., Chest 2007,

132:1733-40). MMP-2 and MMP-12 expression is increased in COPD patients.

In Figure 14, normal human bronchial epithelial (NHBE) cells were transfected for 20 h with 250 nM total of a combination of PDE4B/4D- and PDE7A-specific AON sequences (TOP1572-P²M-7-DAP (SEQ. ID No. 119), TOP1769-P²M-7-DAP (SEQ. ID No. 95) or control sequence (TOP1777s-P²M-7-DAP (SEQ. ID No. 132) and then were stimulated with 10 ng/mL cytomix (TNF- α + IL-1 β + IFN- γ) for 4 h. Results show that gene expression of (D) MCP-1, (E) MMP-2, (F) MMP-12 and (G) IL-1 β is increased following stimulation of NHBE cells.

10 The combination of P²M-DAP modified PDE4B/4D- and PDE7A-specific AON sequences (TOP1572-P²M-7-DAP (SEQ. ID No. 119), TOP1769-P²M-7-DAP (SEQ. ID No. 95) can limit their respective target gene expression (Figure 14A-C) and block the induction of gene expression (Figure 14D-G). The activity of the AON on the expression of MCP-1, MMP-2, MMP-12 and IL-1 β

15 is markedly greater than that of Rolipram, a small molecule PDE4 inhibitor, considering that the concentration of Rolipram used was >2-log higher than that of the AON.

In Figure 15, lung epithelial A549 cells were transfected for 20 h with 238 nM total of a combination of PDE4B/4D- and PDE7A-specific AON sequences (PDE4B/4D; TOP1572 (SEQ. ID No. 42), TOP1572-P²M-7-DAP (SEQ. ID No. 119), PDE7A; TOP1731 (SEQ. ID No. 1), TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP1769-P²M-7-DAP (SEQ. ID No. 95), TOP1777-P²M-7-DAP (SEQ. ID No. 101)) and were stimulated with IL-1 β for 6 h to induce protein secretion of IL-8/CXCL-8 and MCP-1/CCL2. The DAP base and P²M linkage

25 modifications of the AON enhanced the activity of the AON to limit the secretion of the pro-inflammatory chemokines IL-8/CXCL-8 and MCP-1/CCL2 compared to the unmodified (PS-DNA) AON.

**Example 8: In vitro and in vivo response to
P²M-DAP modified AONs**

This example relates to the relative reduction of the immune and inflammatory response of P²M-DAP modified AONs in vitro as well as in vivo following chronic dosing administration to mice. Figure 16 (A, B, C and D) shows the in vitro response of mouse macrophages (RAW 264.7 cells) and human PBMC when exposed for 24 h to either the unmodified PS-DNA sequence TOP1731 (SEQ. ID No. 1), TOP062 (SEQ. ID No. 179) and TOP030 (SEQ. ID No. 161) or the P²M-DAP-modified sequence TOP1731-P²M-7-DAP (SEQ. ID No. 86), TOP062-P²M-11-DAP (SEQ. ID No. 184), TOP062-P²M-12-DAP (SEQ. ID No. 185), TOP057-P²M-4-DAP (SEQ. ID No. 193), TOP057-P²M-5-DAP (SEQ. ID No. 194), TOP030-P²M-15-DAP (SEQ. ID No. 170), TOP031-P²M-1-DAP (SEQ. ID No. 172) or TOP031-P²M-5-DAP (SEQ. ID No. 176). A known immunostimulatory sequence (CpG (SEQ ID No. 133)) was used as positive control. Results indicate that the PS-DNA sequence induced a dose-dependent secretion of TNF- α and IFN- α in RAW 264.7 and PBMC, respectively, while the incorporation of the P²M-DAP modifications completely abrogated this immunostimulatory effect. This result is surprising as the broader activity of known immunostimulatory sequences, such as the CpG sequence (SEQ. ID No. 133) used in this study as positive control, is known to be dependent on the presence of mixed PS/PO linkages (Krieg A. 2002 Annu. Rev. Immunol, 20:709-760).

The response of unmodified PS-DNA and DAP-modified AON sequences was also assessed in vivo following administration by inhalation of 14 consecutive doses of 5 mg/kg to rats (saline was used as control). Lung tissue histopathology was assessed 24 h following the last AON treatment. Figure 17A shows the histopathological changes observed in the animals following treatment with the selected unmodified or DAP-modified AON sequences. Results indicate that animal treated with the unmodified or the DAP-modified AON sequences had comparable type of findings, incidence of findings and

severity of findings clearly showing no benefit of the DAP base modification alone for the tolerability of the AON.

The response of P²M-DAP-modified AON sequences intra-tracheal administration of 10 doses of 2.5 mg/kg to mice (PBS was used as control).
5 Differential cell counts of bronchoalveolar lavage (BAL) and lung tissue histopathology were assessed 24 h following the last AON treatment. Table 8 summarizes the influx of inflammatory cells in BAL and the histopathological assessment of the selected P²M-DAP and unmodified AON sequences (PS-DNA). As shown in Figure 17B, results indicated that animals receiving the
10 P²M-DAP-modified AON (TOP1731-P²M-7-DAP (SEQ. ID No. 86)) had a lower influx of total cells, represented by a decrease in macrophage, lymphocytes and neutrophils, compared to the unmodified PS-DNA sequence TOP1731 (SEQ ID No. 1). Mice treated with the P²M-DAP-modified AON also had a lower incidence of lung tissue findings and the findings observed in
15 these animals were of lesser severity (Grade 1 reflects a minimal severity while Grade 4 reflects severe severity) compared to the unmodified PS-DNA AON (Figure 17C) clearly showing a benefit of the DAP base and P²M linkage modifications for the tolerability of the AON.

Table 8 also summarizes the efficacy/toxicity benefit ratio calculated for the
20 selected P²M-DAP-containing AON sequences. This ratio takes into account the activity (referred to efficacy index or as example here the maximal target knockdown obtained at a given concentration) of the AON vs its relative toxicity (here referred to lung toxicity index or the sum of all the findings and their severity grade). Results in Table 8 show very low ratio values for the
25 unmodified AON sequences (PS-DNA) and higher ratio values (>0.25) for the P²M-DAP-containing AON sequences.

**Example 9: P²M-DAP-modified AON efficacy
in a mouse model of LPS-induced airway inflammation**

This example relates to the efficacy of AON targeting the PDE4B/4D and PDE7A when P²M-DAP modifications are incorporated into the chemistry of the AON in an *in vivo* model of acute lung inflammation in mice. Tables 4c, 4d
5 and 6 describe the compositions of AONs targeting the human or mouse PDE4B/4D and PDE7A and modifications with P²M-DAP.

The activity of P²M-DAP-modified AONs targeting the PDE4B/4D and PDE7A was demonstrated in an *in vivo* model of LPS-induced acute lung
10 inflammation model in mice. In this model of inflammation, mice are exposed to LPS resulting in a marked influx of neutrophils in the lungs of the animals (Figure 18). Neutrophils are a key cell underlying the inflammatory response in many inflammatory diseases such as in COPD. When mice were treated prior to LPS-exposure with a combination (ratio 1:1 w/w) of one modified AON
15 targeting PDE4B/4D (TOP572-P²M-7-DAP (SEQ. ID No.,) or TOP2804-P²M-7-DAP (SEQ. ID No. 138)) and one modified AON targeting PDE7A (TOP1777-P²M-7-DAP (SEQ. ID No. 101) or TOP2707-P²M-7-DAP (SEQ. ID No. 136)), significant reduction of the LPS-induced neutrophil influx was observed (Figure 18A-B). However, when LPS-exposed mice were treated
20 with a control sequence (TOP1777s-P²M-7-DAP (SEQ. ID No. 132), the neutrophil influx to the lung was not reduced (Figure 18A). In mice exposed to LPS but treated with roflumilast, a small molecule inhibitor of PDE4, a dose of 100 mg/kg was required in order to reduce neutrophil influx by a level comparable to that obtained with the AON (Figure 18C).

25 **Example 10: P²M-DAP-modified AON efficacy
in a mouse model of cigarette smoke-induced airway inflammation**

This example relates to the efficacy of AON targeting the PDE4B/4D and PDE7A when P²M-DAP modifications are incorporated into the chemistry of the AON in an *in vivo* model of cigarette smoke-induced lung inflammation in

mice. Tables 4c, 4d and 6 describe the compositions of AONs targeting the human or mouse PDE4B/4D and PDE7A and modifications with P²M-DAP.

The activity of P²M-DAP-modified AONs targeting the PDE4B/4D and PDE7A was also demonstrated in an *in vivo* model of cigarette smoke-induced lung inflammation model in mice. In this model of inflammation, mice are exposed to cigarette smoke for 4 consecutive days resulting in a marked influx of neutrophils in the lungs of animals (Figure 19). When mice were treated every other day prior to smoke exposure with a combination (ratio 1:1 w/w) of one P²M-DAP-modified AON targeting PDE4B/4D (TOP572-P²M-7-DAP (SEQ ID No:) and one P²M-DAP-modified AON targeting PDE7A (TOP1777-P²M-7-DAP (SEQ ID No. 101), a reduction of the total (Figure 19A and B) and the percentage (Figure 19D and E) neutrophil influx was observed. In mice exposed to cigarette smoke but treated with roflumilast, a small molecule inhibitor of PDE4, a dose of 5 mg/kg was required in order to reduce total neutrophil influx (Figure 19C) but with no effect of the percentage of neutrophils (Figure 19F).

The long acting nature of the P²M-DAP-modified AONs was again demonstrated, as they were administered every 2 days compared to roflumilast, which was administered daily.

Although preferred embodiments of the invention have been described herein, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the appended claims. All references cited herein are incorporated by reference.

Table 1.

AON ID	Seq ID No.	Length	AON sequence 5' - 3'	Target site ^a	% PDE7A inhibition ^b
TOP1731	1	21	TCATGAGTGGCAGCTGCAATT	805	>50%
TOP1738	2	19	CAGTCTGATCAGAACTGTC	227	20-50%
TOP1739	3	20	CATCTCCTAGCATACGAATG	253	20-50%
TOP1740	4	19	GGTCCTTACACGTACATC	269	>50%
TOP1741	5	20	GACAGACACTTCAATTTCAG	357	20-50%
TOP1742	6	19	GCTGGAACTTAGTAGCCT	395	<20%
TOP1743	7	18	GTGAAGATCTAAGATATC	414	<20%
TOP1744	8	19	TGAAACCGCAGTACCACGA	339	>50%
TOP1745	9	19	AGGTTAAGCTTACTAGACT	572	20-50%
TOP1746	10	19	TGGAAGTACTCAATTAATC	612	<20%
TOP1747	11	20	CTACGAAGTTTCATCATATC	635	20-50%
TOP1748	12	19	GGACTGCGTTATGGTAAGG	695	20-50%
TOP1749	13	19	CATGGCCTGAGTAACATCC	721	20-50%
TOP1750	14	19	CAGAATTGGCAAGCTTAGG	758	20-50%
TOP1751	15	19	ATGATCCAGATCATGAGTG	817	20-50%
TOP1752	16	19	GTTGCCAAGTAATGGTTAG	867	20-50%
TOP1753	17	19	CAGTACTGAGGTATTCTTG	892	20-50%
TOP1754	18	18	CACTGCAGATCTCCAGTG	920	20-50%
TOP1755	19	19	GAATAAGCCTGATTCTCTC	946	<20%
TOP1756	20	20	GAGCACCTATCTGTGTCTCC	997	20-50%
TOP1757	21	20	GTGGCTAGTATCAGAGCACC	1010	20-50%
TOP1758	22	19	GACTGATGTCTGTGGCTAG	1022	<20%
TOP1759	23	19	GTGTCTGGTGTCTTCTAGG	1096	<20%
TOP1760	24	20	CCACGTCCGACATGGGTTAC	1158	<20%
TOP1761	25	19	TCTATATCTCCTTGATGGA	1221	<20%
TOP1762	26	19	TAGATTCAGTGTGACGATC	1274	<20%
TOP1763	27	19	ACCAATCTGGATGTTGGCA	1294	<20%
TOP1764	28	19	CTGGATGATCCAGATCATG	821	<20%
TOP1765	29	18	GGCTTGTCATTATAATC	482	<20%
TOP1766	30	20	ACATCTCCTAGCATACGAAT	254	<20%
TOP1767	31	21	AAGGTTAAGCTTACTAGACTA	571	20-50%
TOP1768	32	21	GTGCATGGCCTGAGTAACATC	722	20-50%
TOP1769	33	21	ACAGTGCATGGCCTGAGTAAC	725	>50%
TOP1770	34	21	CAGAATTGGCAAGCTTAGGTT	756	20-50%
TOP1771	35	21	TGGCAAGCTTAGGTTCTTTA	750	20-50%
TOP1772	36	20	TGATCCAGATCATGAGTGGC	815	>50%
TOP1773	37	21	TAAAGTTGCCAAGTAATGGTT	869	20-50%
TOP1774	38	21	ACTGAGGTATTCTTGTATAAA	886	20-50%
TOP1775	39	20	GCAGATCTCCAGTGGTGATT	914	20-50%
TOP1776	40	21	CATACACTGGCTTGTCCATT	488	20-50%
TOP1777	41	21	TAAGTAACAGTGCATGGCCTG	731	>50%

^aTarget site on PDE7A1 mRNA sequence (accession # NM_002603)^bPercentage of PDE7A mRNA expression in Flp-In T-Rex 293 cells (260 nM)

Table 2.

AON ID	Seq ID No.	Length	AON sequence 5' - 3'	Target site ^a		% Inhibition ^b	
				PDE4D	PDE4B	PDE4D	PDE4B
TOP1572	42	19	GGTTGCTCAGTCTGCACA	1875	2024	>50%	>50%
TOP1575	43	16	ATCATCCTTTTAACT	937	1086	<20%	<20%
TOP1576	44	16	AGTCCCATTTGTTC	1225	1374	<20%	<20%
TOP1577	45	17	GCCACITCAGCITGGTA	1386	1535	<20%	<20%
TOP1578	46	17	ACCATGTCIATACCAT	1716	1865	<20%	<20%
TOP1579	47	16	TCATITGTTTIGACAT	1746	1895	<20%	<20%
TOP1580	48	17	GAICTTGTCACTTTCTT	1797	1946	<20%	<20%
TOP1581	49	17	GCACAGTGCACCATITT	1863	2012	20-50%	<20%
TOP1582	50	18	GCTCAGTTCIGCACAGTG	1872	2021	<20%	<20%
TOP1583	51	17	TCACACATIGGGCTIAT	1989	2138	<20%	<20%
TOP1584	52	17	TTITCACACATIGGGCT	1992	2141	<20%	<20%
TOP1585	53	17	GCCATTTCACATCAA	475	1037	<20%	<20%
TOP1586	54	17	GGATCCATIGGACTCCG	504	1526	<20%	<20%
TOP1587	55	20	GTCATAITCGCTGTCIGATC	601	2219	<20%	<20%
TOP1588	56	18	GACCTGIGCAAIGGAGT	690	2022	<20%	<20%
TOP1589	57	17	GCCATCTCACTGACIGA	912	1061	<20%	<20%
TOP1590	58	17	CTTTTAACTTGTTIGA	930	1079	<20%	<20%
TOP1591	59	17	TCTAAGAAIGTITTTGA	1017	1166	<20%	<20%
TOP1592	60	17	TGCTTITCTAAGAAIGT	1023	1172	<20%	<20%
TOP1593	61	17	TTCAGTITTIACCTCAA	1174	1323	<20%	<20%
TOP1594	62	17	TGGTCTTCIATAGTCAT	1368	1517	<20%	<20%
TOP1595	63	17	ATCTCCAAATCTGTIAA	1482	1631	<20%	<20%
TOP1596	64	17	AGIATCTCCAAATCTGT	1485	1634	<20%	<20%
TOP1597	65	17	GGATGATCIACITCATG	1527	1676	<20%	<20%
TOP1598	66	17	ACICCAGGATGATCIAC	1533	1682	20-50%	<20%
TOP1599	67	17	GCAAGTTCIGAITTTGT	1572	1721	<20%	<20%
TOP1600	68	18	CTTIGTIGGGTTGCTCAG	1884	2033	<20%	20-50%
TOP1601	69	17	TCACACATIGGGCTIAT	1989	2138	<20%	<20%
TOP1602	70	17	GGATGIACAATITAGTC	2049	2198	<20%	20-50%
TOP1603	71	17	GAIGCCATCTCACTGAC	915	1064	<20%	20-50%
TOP1604	72	17	GTAATGGTCTTCIATAG	1372	1521	<20%	<20%

^aTarget site on PDE4B1 (accession # NM_001037341) or PDE4D1 (accession # NM_001104631) mRNA sequence^bPercentage of PDE4B or PDE4D mRNA expression in Flp-In T-Rex 293 cells (260 nM)

Table 3.

ID	Target(s)	*AON sequence (5'-3')	Number of mismatch(es)			
			Human	Mouse	Rat	Monkey
TOP1731	PDE7A	tc <u>at</u> gagtgggcagctgcaatt	0	2	0	0
TOP1769		acagtgcattggcctgagtaac	0	0	1	0
TOP1777		taagtaacagtgcattggcctg	0	0	0	0
TOP1572	PDE4B/4D	ggttgctcagttctgcaca	0	0	0	0

* Mismatch(es) with animal species are indicated in bold underlined letters

5 Table 4.

Table 4a.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^c
TOP1731	1	PS-tcatgagtgaggcagctgcaatt	100	N.A
TOP1731-F3	73	PS-tcatgagtgaggcagctgcaatt	100	+21
TOP1731-DAP	74	PS-tcxtgxtggcagctgcxxtt	100	-1
TOP1731-PO	75	PO-tcatgagtgaggcagctgcaatt	0	-77
TOP1731-PO-DAP	76	PO-tcxtgxtggcagctgcxxtt	0	-66
TOP1731-PO-OMe1	77	PO-TCATGagtgaggcagctgCAATT	0	-168
TOP1731-P ² M-1	78	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	85	+7
TOP1731-P ² M-2	79	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	65	-55
TOP1731-P ² M-3	80	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	50	-30
TOP1731-P ² M-3-DAP	81	t _s C _s X _s t _s G _s X _s G _s t _s G _s G _s C _s X _s G _s C _s X _s X _s t _s t	50	-21
TOP1731-P ² M-4	82	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	35	-64
TOP1731-P ² M-5	83	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	70	+4
TOP1731-P ² M-6	84	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	60	-29
TOP1731-P ² M-7	85	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	60	+9
TOP1731-P ² M-7-DAP	86	t _s C _s X _s t _s G _s X _s G _s t _s G _s G _s C _s X _s G _s C _s X _s X _s t _s t	60	+21
TOP1731-P ² M-8	87	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	25	-79
TOP1731-P ² M-8-DAP	88	t _s C _s X _s t _s G _s X _s G _s t _s G _s G _s C _s X _s G _s C _s X _s X _s t _s t	25	-40
TOP1731-P ² M-9	89	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	40	-46
TOP1731-P ² M-9-DAP	90	t _s C _s X _s t _s G _s X _s G _s t _s G _s G _s C _s X _s G _s C _s X _s X _s t _s t	40	-30
TOP1731-P ² M-10	91	t _s C _s a _s t _s G _s a _s G _s t _s G _s G _s C _s a _s G _s C _s a _s a _s t _s t	50	-21
TOP1731-P ² M-10-DAP	92	t _s C _s X _s t _s G _s X _s G _s t _s G _s G _s C _s X _s G _s C _s X _s X _s t _s t	50	+8
TOP1731-LNA1	93	PS-tcatggtggcagctgcaatt	100	N.A

Table 4b.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^c
TOP1769	33	PS-acagtgcattggcctgagtaac	100	N.A
TOP1769-P ² M-7	94	a ₅ C ₈ a ₀ G ₀ t ₅ G ₈ C ₀ a ₀ t ₅ G ₈ C ₀ C ₀ t ₅ G ₈ a ₅ G ₀ t ₀ a ₈ a ₅ C	60	+37
TOP1769-P ² M-7-DAP	95	X ₈ C ₈ X ₀ G ₀ t ₅ G ₈ C ₀ X ₀ t ₅ G ₈ C ₀ C ₀ t ₅ G ₈ X ₈ G ₀ t ₀ X ₈ X ₈ C	60	+43
TOP1769-P ² M-9	96	a ₅ C ₈ a ₀ G ₀ t ₅ G ₈ C ₀ a ₈ t ₀ G ₀ C ₀ C ₈ t ₅ G ₀ a ₀ G ₀ t ₀ a ₈ a ₅ C	67	+2
TOP1769-P ² M-9-DAP	97	X ₈ C ₈ X ₀ G ₀ t ₅ G ₈ C ₀ X ₈ t ₀ G ₀ C ₀ C ₈ t ₅ G ₀ X ₀ G ₀ t ₀ X ₈ X ₈ C	67	-9
TOP1769-P ² M-10	98	a ₅ C ₈ a ₀ G ₀ t ₅ G ₈ C ₀ a ₀ t ₅ G ₈ C ₀ C ₀ t ₅ G ₈ a ₅ G ₀ t ₀ a ₈ a ₅ C	50	+11
TOP1769-P ² M-10-DAP	99	X ₈ C ₈ X ₀ G ₀ t ₅ G ₈ C ₀ X ₀ t ₅ G ₈ C ₀ C ₀ t ₅ G ₈ X ₈ G ₀ t ₀ X ₈ X ₈ C	50	+27

Table 4c.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^c
TOP1777	41	PS-taagtaacagtgcattggcctg	100	N.A
TOP1777-P ² M-7	100	t ₈ a ₈ a ₀ G ₀ t ₅ a ₈ a ₀ C ₀ a ₈ G ₈ t ₅ G ₀ C ₀ a ₈ t ₅ G ₈ C ₀ C ₈ t ₅ G	60	+13
TOP1777-P ² M-7-DAP	101	t ₈ X ₈ X ₀ G ₀ t ₅ X ₈ X ₀ C ₀ X ₈ G ₈ t ₅ G ₀ C ₀ X ₈ t ₅ G ₈ C ₀ C ₈ t ₅ G	60	+29
TOP1777-P ² M-9	102	t ₈ a ₈ a ₀ G ₀ t ₅ a ₈ a ₀ C ₀ a ₈ G ₈ t ₅ G ₀ C ₀ C ₈ a ₈ t ₀ G ₀ C ₀ C ₈ t ₅ G	67	-31
TOP1777-P ² M-9-DAP	103	t ₈ X ₈ X ₀ G ₀ t ₅ X ₈ X ₀ C ₀ X ₈ G ₈ t ₅ G ₀ C ₀ X ₈ t ₅ G ₈ C ₀ C ₈ t ₅ G	67	-13
TOP1777-P ² M-10	104	t ₈ a ₈ a ₀ G ₀ t ₅ a ₈ a ₀ C ₀ a ₈ G ₈ t ₅ G ₀ C ₀ a ₈ t ₅ G ₈ C ₀ C ₈ t ₅ G	50	-47
TOP1777-P ² M-10-DAP	105	t ₈ X ₈ X ₀ G ₀ t ₅ X ₈ X ₀ C ₀ X ₈ G ₈ t ₅ G ₀ C ₀ X ₈ t ₅ G ₈ C ₀ C ₈ t ₅ G	50	-8

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Table 4d.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^c	
				PDE4D	PDE4B
TOP1572	42	PS-ggttgctcagitctgcaca	100	N.A.	N.A
TOP1572-F2	106	PS-ggttgctcagitctgcaca	100	+5	+20
TOP1572-DAP	107	PS-ggttgctcxgitctgcxcx	100	-1	-3
TOP1572-PO	108	PO-ggttgctcagitctgcaca	0	-58	-113
TOP1572-PO-DAP	109	PO-ggttgctcxgitctgcxcx	0	-24	+3
TOP1572-PO-OMe1	110	PO-GGTGctcagitctGCACA	0	-47	-112
TOP1572-P ² M-1	112	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₈ C ₈ a ₈ C ₈ a	83	-4	-4
TOP1572-P ² M-2	112	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₈ C ₈ a ₈ C ₈ a	56	-22	-37
TOP1572-P ² M-3	113	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₈ C ₈ a ₈ C ₈ a	50	-53	-25
TOP1572-P ² M-3-DAP	114	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₀ X ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₈ C ₈ X ₈ C ₈ X	50	-20	+9
TOP1572-P ² M-4	115	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₈ C ₈ a ₈ C ₈ a	39	+2	-26
TOP1572-P ² M-5	116	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₈ a ₈ G ₈ i ₈ t ₅ C ₀ t ₅ G ₀ C ₈ a ₈ C ₈ a	67	-16	-36
TOP1572-P ² M-6	117	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₈ a ₈ G ₈ i ₈ t ₅ C ₀ t ₅ G ₀ C ₈ a ₈ C ₈ a	56	-12	-35
TOP1572-P ² M-7	118	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ a ₈ C ₈ a	56	-9	-1
TOP1572-P ² M-7-DAP	119	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ X ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ X ₈ C ₈ X	56	0	-3
TOP1572-P ² M-8	120	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ a ₈ C ₈ a	28	-61	-82
TOP1572-P ² M-8-DAP	121	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ X ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ X ₈ C ₈ X	28	-27	-43
TOP1572-P ² M-9	122	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ a ₈ C ₈ a	44	-32	-36
TOP1572-P ² M-9-DAP	123	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ X ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ X ₈ C ₈ X	44	-19	-16
TOP1572-P ² M-10	124	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₈ a ₈ G ₈ i ₈ t ₅ C ₀ t ₅ G ₀ C ₈ a ₈ C ₈ a	56	-49	-64
TOP1572-P ² M-10-DAP	125	G ₈ G ₈ t ₅ t ₅ G ₈ C ₈ t ₅ C ₀ C ₈ X ₈ G ₈ i ₈ t ₅ C ₀ t ₅ G ₀ C ₈ X ₈ C ₈ X	56	-22	-23
TOP1572-P ² M-11	126	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ a ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ a ₈ C ₈ a	50	-35	-52
TOP1572-P ² M-11-DAP	127	G ₈ G ₈ t ₀ t ₀ G ₈ C ₈ t ₀ C ₀ X ₀ G ₀ i ₀ t ₅ C ₈ t ₅ G ₀ C ₀ X ₈ C ₈ X	50	-20	-30

^aPercentage of phosphorothioate linkage in AON sequence.^cPercentage of difference compared to PDE mRNA inhibition in Flp-In T-Rex 293 cells treated with 236nM PS-DNA.

Sugar Chemistry	Symbol:
DNA	lower case
FANA	bold lowercase
2'-OMe-RNA	BOLD UPPERCASE
LNA	<i>Underlined italic lowercase</i>
Linkage Chemistry	Symbol:
All Phosphodiester (PO)	Denote sequence with prefix PO-
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Mixed PO/PS linkages (P ² M)	Denote PS with s subscript and PO with o subscript
Base Chemistry	Symbol:
inosine	i
2-amino-2'-deoxyadenosine	<u>x</u>

5 **Table 5.**

AON ID	Seq ID No.	Sequence (5'-3')	% PS^a
TOP1731s	128	PS-agtactcaccgtcgcgcttaa	100
TOP1769s	129	PS-tgtcacgtaccggactcattg	100
TOP1777s	130	PS-attcattgtcacgtaccggac	100
TOP1572s	131	PS-ccaacgagtciaacgtgt	100
TOP1777s-P ² M-7-DAP	132	x _s t _s t _o C _o x _s t _o g _o t _s C _s x _s C _o g _o t _s x _s C _o g _o g _s x _s C	60
TOP1731s-P ² M-7-DAP	155	x _s g _s t _o x _o C _s t _s C _o x _o C _s g _s t _o C _o g _s x _s C _o g _o t _s x _s x	60
TOP1572s-P ² M-7-DAP	156	C _s C _s x _o C _o g _s x _o g _o t _s C _s i _o x _o g _s x _s C _o g _o t _s g _s t	56
CpG	133	g _s g _s g _o g _o a _o C _o g _o a _o t _o C _o g _o t _o C _o g _s g _s g _s g	37

^aPercentage of phosphorothioate linkage in AON sequence.

Sugar Chemistry	Symbol:
DNA	lower case
Linkage Chemistry	Symbol:
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Mixed PO/PS linkages (P ² M)	Denote PS with s subscript and PO with o subscript
Base Chemistry	Symbol:
inosine	i
2-amino-2'-deoxyadenosine	<u>x</u>

10

Table 6.

AON ID	Seq ID No.	Sequence (5'-3')	% PS^a
TOP2707-F3	134	PS-tccagatcgtgagtggc	100
TOP2707Mi2-F3	135	PS-tgctcaacgtctgaggg	100
TOP2707-P ² M-7-DAP	136	t _s C _s C _o x _o g _o x _s t _s C _o g _o t _o g _s x _s g _o t _o g _s C	50
TOP2804-F1	137	PS-ggttgctcaggtctgcacag	100
TOP2804-P ² M-7-DAP	138	g _s g _s t _o t _o g _s C _s t _o C _o x _s g _s g _o t _o C _s t _s g _o C _o x _s C _s x _s g	58

^aPercentage of phosphorothioate linkage in AON sequence.

Sugar Chemistry	Symbol:
DNA	lower case
FANA	bold lowercase
Linkage Chemistry	Symbol:
All Phosphodiester (PO)	Denote sequence with prefix PO-
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Mixed PO/PS linkages (P ² M)	Denote PS with s subscript and PO with o subscript
Base Chemistry	Symbol:
2-amino-2'-deoxyadenosine	<u>x</u>

5 Table 7.

Target gene	Sense/Antisense primer sequences (5'-3')	SEQ ID No.
PDE7A	TCAGGCCATGCACTGTTACT	139
	CCTGATTCTCTCAATAAGCCC	140
PDE4B	TGGCAGACCTGAAGACAATG	141
	AAATTCCTCCATGATGCGG	142
PDE4D	CAGAATATGGTGCACTGTGC	143
	AGTCTATGAAGCCACCTGTG	144
MCP-1	CAATAGGAAGATCTCAGTGC	145
	GTGTTCAAGTCTTCGGAGTT	146
MMP-2	AGGGCACATCCTATGACAGC	147
	ATTGTTGCCCAGGAAAGTG	148
MMP-12	CCAGTGACAAAAATGAAATATAGTGGA	149
	GACTCCACATCGAGGTGCGT	150
IL-1 β	CAGGGACAGGATATGGAGCAA	151
	GCAGACTCAAATTCAGCTTGTTA	152
B2M	CAAGGACTGGTCTTTCTATCTCTTGT	153
	GTGGAGCAACCTGCTCAGATAC	154

Table 8.

	PBS	TOP1572	TOP1572-P ² M-7	TOP1572-P ² M-7-DAP	TOP1731	TOP1731-P ² M-7-DAP	TOP1769-P ² M-7-DAP	TOP1777-P ² M-7-DAP
Dose (mg/kg)	-	2.5	2.5	2.5	2.5	2.5	2.5	2.5
BAL								
Total cells	2.7x10 ⁵ (6.4x10 ⁴)	1.1x10 ⁶ (2.7x10 ⁵)	1.6x10 ⁵ (3.5x10 ⁴)	3.4x10 ⁵ (7.9x10 ⁴)	4.0x10 ⁶ (1.1x10 ⁶)	3.0x10 ⁵ (5.5x10 ⁴)	3.1x10 ⁵ (9.7x10 ⁴)	2.7x10 ⁵ (1.7x10 ⁵)
Neutrophils	4.3x10 ³ (2.7x10 ³)	8.4x10 ⁴ (6.9x10 ⁴)	5.7x10 ³ (2.0x10 ³)	1.7x10 ⁴ (5.3x10 ³)	8.9x10 ⁵ (6.2x10 ⁵)	3.9x10 ³ (2.6x10 ³)	2.2x10 ⁴ (1.1x10 ⁴)	3.2x10 ³ (2.6x10 ³)
Macrophage	2.6x10 ⁵ (5.8x10 ⁴)	9.1x10 ⁵ (3.1x10 ⁵)	1.4x10 ⁵ (3.6x10 ⁴)	3.1x10 ⁵ (8.1x10 ⁴)	2.4x10 ⁶ (4.7x10 ⁵)	3.0x10 ⁵ (7.0x10 ⁴)	3.0x10 ⁵ (8.7x10 ⁴)	1.3x10 ⁵ (2.4x10 ⁴)
Lymphocytes	8.1x10 ³ (1.1x10 ⁴)	1.9x10 ⁵ (5.4x10 ⁴)	1.1x10 ⁴ (3.2x10 ³)	1.1x10 ⁴ (5.0x10 ³)	6.8x10 ⁵ (2.5x10 ⁵)	9.4x10 ³ (3.8x10 ³)	2.0x10 ⁴ (1.5x10 ⁴)	3.2x10 ⁴ (1.4x10 ⁴)
% Large macrophages	6.0 (1.8)	61.0 (4.3)	33.0 (8.5)	18.0 (4.4)	55.0 (4.1)	37.5 (5.2)	18.2 (5.4)	15.7 (5.9)
LUNG								
Infiltrate, mixed cell, perivascular								
Grade 1	-	1/4	-	-	1/4	-	-	-
Grade 2	-	1/4	-	-	1/4	-	-	-
Grade 3	-	2/4	-	-	2/4	-	-	-
Inflammation, alveolar (chronic active)								
Grade 1	-	-	-	-	-	1/4	-	-
Grade 2	-	1/4	-	-	1/4	-	-	-
Grade 3	-	1/4	-	-	3/4	-	-	-
Grade 4	-	2/4	-	-	-	-	-	-
Infiltrate, macrophages alveolar								
Grade 1	-	-	-	4/4	-	3/4	1/4	2/4
Grade 2	-	-	-	-	4/4	-	3/4	-
Lung Toxicity Index*								
	-	5.5	0	1	7	1	1.75	0.5
Efficacy Index**								
	-	75% ^A	50% ^A	71% ^A	70%	91%	89%	66%
Efficacy/Toxicity Ratio***								
	-	0.14	>10	0.71	0.1	0.91	0.51	1.32

BAL: values represent mean (±SEM)

LUNG: values represent number of animals with finding/total animals

A: mean efficacy for the two targets (PDE4B and PDE4D)

*Lung Toxicity Index = $\sum(\text{finding}\% \times \text{Severity Grade})$

For example for TOP1572:

Lung Toxicity Index = $(0.25 \times 1 + 0.25 \times 2 + 0.5 \times 3) + (0.25 \times 2 + 0.25 \times 3 + 0.5 \times 4) = 5.5$

**Efficacy Index = % maximal target knockdown in vitro

*** Efficacy/Toxicity Ratio = Efficacy Index/Lung Toxicity Index

Table 9.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a
TOP004	157	PS-gggctctgcxgcgggxtggt	100
TOP005	158	PS-gttxtctxcttccxctgctg	100
TOP006	159	PS-tggcactttagtggtgctg	100
TOP007	160	PS-actcatattcatagggtg	100

^aPercentage of phosphorothioate linkage in AON sequence.

Sugar Chemistry	Symbol:
DNA	lower case
Linkage Chemistry	Symbol:
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Base Chemistry	Symbol:
2-amino-2'-deoxyadenosine	x

5

Table 10

Table 10a.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^a
TOP030	161	PS-cacctctgtcaccagcatg	100	N.A
TOP030-P ² M-7-DAP	162	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _s X _o C _o C _s X _o G _o C _o X _s T _e G	56	-47
TOP030-P ² M-8-DAP	163	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _o C _s C _s X _o G _o C _o X _s T _e G	56	-14
TOP030-P ² M-9-DAP	164	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _s C _s C _o X _o G _o C _o X _s T _e G	50	-16
TOP030-P ² M-10-DAP	165	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _s C _s C _o X _o G _o C _o X _s T _e G	50	-35
TOP030-P ² M-11-DAP	166	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _s X _o C _s C _s X _o G _o C _o X _s T _e G	78	-4
TOP030-P ² M-12-DAP	167	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _s C _s C _o X _o G _o C _o X _s T _e G	67	-33
TOP030-P ² M-13-DAP	168	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _s C _s C _s X _o G _o C _o X _s T _e G	67	-25
TOP030-P ² M-14-DAP	169	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _o X _s C _s C _s X _o G _o C _o X _s T _e G	78	-51
TOP030-P ² M-15-DAP	170	C _s X _s C _o C _o T _e C _s T _e G _o T _e C _s X _o C _s C _o X _o G _o C _o X _s T _e G	67	-11
TOP031	171	PS-gtacttccggaacctctctcc	100	N.A
TOP031-P ² M-1-DAP	172	G _s T _s X _o C _o T _e T _s C _o C _o G _s G _s X _o X _o C _s C _o C _o T _e C _s C _s C	60	+2
TOP031-P ² M-2-DAP	173	G _s T _s X _o C _o T _e T _s C _o C _o G _o G _o X _o X _s C _s C _o C _o T _e C _s C _s C	60	-2
TOP031-P ² M-3-DAP	174	G _s T _s X _o C _o T _e T _s C _o C _o G _s G _s X _s X _s C _s C _o C _o T _e C _s C _s C	70	+9
TOP031-P ² M-4-DAP	175	G _s T _s X _s C _o T _e T _s C _o C _o G _s G _s X _o X _o C _s C _o T _e C _s C _s C	80	+14
TOP031-P ² M-5-DAP	176	G _s T _s X _s C _o T _e T _s C _s C _s G _s G _o X _o X _s C _s C _o C _o T _e C _s C _s C	70	+6
TOP031-P ² M-6-DAP	177	G _s T _s X _s C _s T _e T _s C _s C _o G _o G _o X _o X _o C _o C _s T _e C _s C _s C	70	-1
TOP031-P ² M-7-DAP	178	G _s T _s X _s C _s T _e T _s C _s C _s G _o G _o X _o X _o C _s C _s T _e C _s C _s C	80	-1

10

^aPercentage of phosphorothioate linkage in AON sequence.

Sugar Chemistry	Symbol:
DNA	lower case
FANA	bold lowercase
Linkage Chemistry	Symbol:
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Mixed PO/PS linkages (P ² M)	Denote PS with s subscript and PO with o subscript
Base Chemistry	Symbol:
2-amino-2'-deoxyadenosine	x

Table 10b.

AON ID	Seq ID No.	Sequence (5'-3')	% PS ^a	% Difference vs PS-DNA ^c
TOP062	179	PS-ctctccactttccacggcctg	100	N.A
TOP062-P ² M-7-DAP	180	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	58	-29
TOP062-P ² M-8-DAP	181	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	53	-41
TOP062-P ² M-9-DAP	182	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	53	-26
TOP062-P ² M-10-DAP	183	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	42	-27
TOP062-P ² M-11-DAP	184	C _s T _s C _s T _o C _s C _s X _o C _o T _s C _s C _s X _s C _o G _o G _o C _s C _s T _s G	79	+2
TOP062-P ² M-12-DAP	185	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _s C _s X _s C _o G _o G _o C _s C _s T _s G	68	-15
TOP062-P ² M-13-DAP	186	C _s T _s C _s T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	68	-20
TOP062-P ² M-14-DAP	187	C _s T _s C _s T _o C _s C _s X _o C _o T _s C _o C _o X _s C _o G _o G _o C _s C _s T _s G	79	-15
TOP062-P ² M-15-DAP	188	C _s T _s C _o T _o C _s C _s X _o C _o T _s C _s C _s X _s C _o G _o G _o C _s C _s T _s G	68	-8
TOP057	189	PS-gaccgagctggccacctcc	100	N.A.
TOP057-P ² M-1-DAP	190	G _s X _s C _o C _o G _o X _s G _o C _o T _s G _o C _o C _s X _s C _o C _o T _s C _s C	56	-34
TOP057-P ² M-2-DAP	191	G _s X _s C _s C _o G _o X _s G _s C _o T _s G _o C _s C _s X _s C _o C _o T _s C _s C	56	-32
TOP057-P ² M-3-DAP	192	G _s X _s C _o C _o G _o X _s G _s C _s T _s G _o G _s C _s C _s X _s C _o C _o T _s C _s C	67	-4
TOP057-P ² M-4-DAP	193	G _s X _s C _s C _o G _o X _s G _s C _o T _s G _o G _s C _o C _s X _s C _s C _o T _s C _s C	78	+5
TOP057-P ² M-5-DAP	194	G _s X _s C _s C _o G _o X _s G _s C _s T _s G _o G _s C _s C _s X _s C _o C _o T _s C _s C	67	-16
TOP057-P ² M-6-DAP	195	G _s X _s C _s C _s G _s X _s G _o C _o T _s G _o C _o C _s X _s C _s C _s T _s C _s C	67	-3
TOP057-P ² M-7-DAP	197	G _s X _s C _s C _s G _s X _s G _s C _o T _s G _o C _s C _s X _s C _s C _s T _s C _s C	78	+3

5 ^aPercentage of phosphorothioate linkage in AON sequence.

Sugar Chemistry	Symbol:
DNA	lower case
FANA	bold lowercase
Linkage Chemistry	Symbol:
All Phosphorothioate (PS)	Denote sequence with prefix PS-
Mixed PO/PS linkages (P ² M)	Denote PS with s subscript and PO with o subscript
Base Chemistry	Symbol:
2-amino-2'-deoxyadenosine	<u>x</u>

CLAIMS

1. An oligonucleotide directed against a target gene, wherein the oligonucleotide is capable of hybridizing to at least a portion of a nucleic acid sequence encoding the gene under stringent conditions, and
5 wherein,

at least one nucleotide of the oligonucleotide is 2-amino-2'-deoxyadenosine (DAP); and

the internucleoside linkages of the oligonucleotide comprises at least three alternating segments, each segment consisting of either at least
10 one phosphorothioate or at least one phosphodiester bond.
2. The oligonucleotide of claim 1, wherein the target gene is the common beta sub-unit of IL-3, IL-5 and GM-CSF.
3. The oligonucleotide of claim 1, wherein the target gene is CCR3 chemokine receptor.
- 15 4. The oligonucleotide of claim 1, wherein the target gene is a target Phosphodiesterase (PDE).
5. The oligonucleotide of claim 4, wherein the target PDE is selected from the group consisting of PDE3A, PDE3B, PDE4A, PDE4B, PDE4C, PDE4D, PDE7A1, PDE7A2, PDE7A3 and PDE7B.
- 20 6. The oligonucleotide of claim 5, wherein the target PDE is PDE7A.
7. The oligonucleotide of claim 5, wherein the target PDE is PDE4B.
8. The oligonucleotide of claim 5, wherein the target PDE is PDE4D.
9. The oligonucleotide of any one of claims 1-8, wherein the ratio of phosphorothioate to phosphodiester bonds is between 30:70 and 70:30.

10. The oligonucleotide of claim 9, wherein the ratio of phosphorothioate to phosphodiester bonds is between 40:60 and 60:40.
11. The oligonucleotide of claim 10, wherein the ratio of phosphorothioate to phosphodiester bonds is between 45:55 and 55:45.
- 5 12. The oligonucleotide of claim 11, wherein the ratio of phosphorothioate to phosphodiester bonds is about 50:50.
13. The oligonucleotide of any one of claims 1-12, wherein the oligonucleotide is at least 80% complementary to the target gene.
14. The oligonucleotide of claim 13, wherein the oligonucleotide is 100%
10 complementary to the target gene.
15. The oligonucleotide of any one of claims 1-14, wherein the efficacy/toxicity ratio is greater than 0.25.
16. The oligonucleotide of any one of claims 1-14, wherein the oligonucleotide is between 15-25 nucleotides in length.
- 15 17. The oligonucleotide of claim 16, wherein the oligonucleotide is between 18-22 nucleotides in length.
18. The oligonucleotide of claim 4, having a base sequence selected from the group consisting of SEQ ID NOs. 1-72, preferably SEQ ID NOs. 1, 4, 8, 33, 36, 41 and 42, wherein at least one adenosine is replaced with
20 DAP.
19. The oligonucleotide of claim 18, wherein the oligonucleotide has a base sequence selected from the group consisting of SEQ ID NOs. 1, 33, 41 and 42.

20. The oligonucleotide of claim 4, comprising any one of SEQ ID NOs. 81, 86, 90, 92, 95, 97, 99, 101, 103, 105, 114, 119, 121, 123, 125 and 127, preferably SEQ ID NOs. 86, 95, 101 and 119.
21. The oligonucleotide of claim 20, consisting of any one of SEQ ID NOs. 81, 86, 90, 92, 95, 97, 99, 101, 103, 105, 114, 119, 121, 123, 125 and 127, preferably SEQ ID NOs. 86, 95, 101 and 119.
22. The oligonucleotide of claim 2, having a base sequence selected from the group consisting of SEQ ID NOs. 179 and 189, wherein at least one adenosine is replaced with DAP.
23. The oligonucleotide of claim 22, comprising any one of SEQ ID NOs. 180-188 and 190-197.
24. The oligonucleotide of claim 3, having a base sequence selected from the group consisting of SEQ ID NOs. 161 and 171, wherein at least one adenosine is replaced with DAP.
25. The oligonucleotide of claim 24, comprising any one of SEQ ID NOs. 162-170 and 172-178.
26. A pharmaceutical composition comprising the oligonucleotide of any one of claims 1-25 and a pharmaceutically acceptable carrier.
27. The pharmaceutical composition of claim 17 comprising at least two oligonucleotides of any one of claims 1-25.
28. The pharmaceutical composition of claim 27, wherein the at least two oligonucleotides are directed against PDE7A and PDE4B respectively.
29. The pharmaceutical composition of claim 27, wherein the at least two oligonucleotides are directed against PDE7A and PDE4D respectively.

30. The pharmaceutical composition of any one of claims 28 and 29, wherein the oligonucleotide directed against PDE4B or PDE4D also downregulates the other.
- 5 31. The pharmaceutical composition of claim 27, wherein the at least two oligonucleotides are directed against the common beta sub-unit of IL-3, IL-5 and GM-CSF, and CCR3 chemokine receptor respectively.
32. The pharmaceutical composition of any one of claims 26-31, for the treatment of inflammation.
- 10 33. The pharmaceutical composition of any one of claims 26-31, for the treatment of respiratory disease.
- 15 34. The pharmaceutical composition of claim 33, for the treatment of at least one of chronic obstructive pulmonary disease, asthma, eosinophilic cough, bronchitis, acute and chronic rejection of lung allograft, sarcoidosis, pulmonary fibrosis, rhinitis, sinusitis, viral infection or a neoplastic disease.
35. A method of treating respiratory disease in a subject comprising administering the pharmaceutical composition of any one of claims 26-31.
- 20 36. Use of the pharmaceutical composition of any one of claims 26-31 in the preparation of a medicament for the treatment of respiratory disease.
37. Use of the pharmaceutical composition of any one of claims 26-31 for the treatment of respiratory disease.
- 25 38. An oligonucleotide comprising the base sequence of any one of SEQ ID NOs. 1-72, 161, 171, 179 and 189, preferably SEQ ID NOs. 1, 4, 8, 33, 36, 41, 42, 161, 171, 179 and 189.

39. The oligonucleotide of claim 38, wherein at least one adenosine is replaced with DAP.
40. An oligonucleotide consisting of any one of SEQ ID NOs. 1-127 and 161-197.