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(54) Title: NOVEL COMPOSITION FOR THE TREATMENT OF CYSTIC FIBROSIS

(57) Abstract: The present invention provides a composition comprising at least one prostacyclin orprostacyclin analogue or a pharmaceutically acceptable salt thereof and at least one compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin or a pharmaceutically acceptable salt thereof for use in preventing or treating cystic fibrosis. The invention also provides a composition comprising Treprostiniland a compound from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycinor a pharmaceutically acceptable salt thereof.

# Novel composition for the treatment of Cystic Fibrosis

The present invention provides compositions comprising at least one prostacyclin or prostacyclin analogue and at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin for use in preventing or treating cystic fibrosis as well as specific compositions comprising combinations of said compounds.

Cystic fibrosis (CF) is a genetic disease resulting from mutations in a 230 kb gene on chromosome 7 encoding a 1480 amino acid polypeptide known as the cystic fibrosis transmembrane conductance regulator (CFTR) which serves as a chloride channel in epithelial membranes. Over 1000 mutant alleles have been identified to date. The most common mutation, ΔF508, is the deletion of a phenylalanine residue at codon 508 in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This mutation results in a severe reduction in CFTR function, and leads to the classic cystic fibrosis phenotype characterized with abnormality in exocrine gland functions like raised sweat chloride, recurrent respiratory infection with bronchiectasis, and early-onset of pancreatic insufficiency.

Clinically, CF is usually suspected when one or more typical CF phenotypic features are present in a subject. This could be a chronic pulmonary disease alone or very often associated with gastrointestinal and nutritional abnormalities (e.g. pancreatic insufficiency and recurrent pancreatitis), salt loss syndromes and male urogenital abnormalities (i.e. obstructive azoospermia). In the human lung, thick, tenacious secretions obstruct the distal airways and submucosal glands, which express CFTR. Ductular dilatation of these glands (associated with blockage by mucus) and the plastering of airway surfaces by thick, viscous, neutrophil dominated mucopurulent debris are among the pathological hallmarks of the disease. Pulmonary inflammation is another major cause of the decline in respiratory function in subjects with cystic fibrosis and may precede the onset of chronic infection. Mucinous impaction and thick concretions within pancreatic ducts lead to chronic fibrosis, fatty replacement of the gland, or both in a large subgroup of subjects with a previous diagnosis of idiopathic or alcoholic pancreatitis.

Cystic fibrosis is the most common fatal inherited disease in the Caucasian population, affecting about 4 in 10,000 children. In the United States, the median age at death has increased from 8.4 years of age in 1969 to 14.3 years of age in 1998.

The mean age of death has increased from 14 years in 1969 to 32.4 years of age in 2003 (Cystic Fibrosis Foundation). For children born in the 1990s, the median survival is predicted to be over 40 years. A major contributor to the significant increase in life expectancy is improved treatment of chronic respiratory tract infections and elimination of mucus in CF subjects as well as improved nutrition and earlier diagnosis.

Loss of the cystic fibrosis transmembrane conductance regulator (CFTR) anion conductance from the apical membranes of airway epithelia disrupts regulation of the airway surface liquid layer. This leads to impaired mucociliary clearance, airway infection, and inflammation characteristic of cystic fibrosis (CF). The common ΔF508 mutation of CFTR is present on at least one allele in >90% of CF patients, and >50% of patients are homozygous for ΔF508, the rest being compound heterozygous. A central issue in CF disease is the inability of this common CFTR variant to achieve the native, folded state that will exit from the endoplasmic reticulum (ER) and traffic to the epithelial cell apical membrane.

If acquisition of the native conformation is retarded, CFTR is thought to maintain excessive or prolonged interactions with molecular chaperones, which then target the protein for degradation by mechanisms that police the ER for misfolded or incompletely complexed proteins. ER-associated degradation (ERAD) involves ubiquitination of aberrant proteins and their delivery to the proteasome for digestion. If ERAD lags behind the rate of protein synthesis, or during treatment with proteasome inhibitors, aggregates of the mutant protein accumulate. CFTR was the first integral membrane mammalian protein to be identified as a substrate for ubiquitin-proteasome mediated degradation, and it has served as a model for the growing list of diseases of protein conformation, which account for a diverse set of pathological etiologies.

Essentially all of the ΔF508 CFTR produced by the cell is destroyed by ERAD. Also, due to its complex folding pattern, 60–70% of the wild-type (wt) protein may be similarly degraded, although this may vary among cell types. The proteolytic cleavage patterns of the immature forms of wt and ΔF508 CFTR are similar, whereas the digestion pattern of mature wt CFTR is different. This finding supports the concept that at least a portion of the ER-retained mutant CFTR is present in an intermediate conformation that is formed along the normal CFTR folding pathway, as opposed to the formation of a variant protein structure. For ΔF508 CFTR, this

intermediate conformation cannot proceed beyond a critical step in the folding process, but this implies that ΔF508 CFTR could be rescued if it were possible to facilitate this step.

A variety of experimental conditions, such as reduced temperature, incubation with chemical chaperones, or pharmacological correctors, can promote the escape of ΔF508 CFTR from the ER, yielding a functional anion channel at the cell surface. In addition, investigators have reported restoration of ΔF508 CFTR function by coexpression of various partial CFTR constructs or subdomains from wt CFTR. However, a consensus as to which CFTR subdomains are effective in mutant protein rescue is not apparent, and the mechanism of this effect remains obscure. In addition, CFTR fragment-induced rescue has been observed primarily in cells exogenously overexpressing both the CFTR fragment and full-length ΔF508 CFTR.

Prostaglandin I2 (prostacyclin; epoprostenol, PGI2) is an oxygenated metabolite of arachidonic acid formed enzymatically by the sequential activities of cyclooxygenase and PGI synthase enzymes. It is produced constitutively by vascular endothelial and smooth muscle cells and is induced under inflammatory conditions in vascular cells and macrophages.

Cystic fibrosis is unrelated to pulmonary fibrosis because it is a disease that originates in the bronchial epithelium. Because of the absence of CFTR, there is too little water in the mucus that covers the bronchial epithelium; accordingly, the cilia cannot move the thick mucus and mucociliary clearance breaks down (mucociliary clearance works like a conveyor belt, where the cilia beat rhythmically in a concentrated manner to move the mucus back to the trachea and pharynx, from where it may be cleared by swallowing or coughing etc.). If mucociliary breaks down, the bacteria cannot be removed from the bronchi, the bronchi are colonized by bacteria and there are repeated bouts of lung infections that destroy the lung. The situation can be remedied by restoring Cl- fluxes to the bronchial epithelium. Thus, in cystic fibrosis the site of action is the airway epithelium of the bronchi. The site of action is anatomically distinct (lung interstitium vs. bronchial airway), involves a different set of cells (fibroblasts, vascular smooth muscle cells, endothelium versus absorbing and secreting bronchial epithelial cells) and presumably also involves different receptors (prostacyclin receptor vs. possibly EP2-receptor).

PGI2 is a potent vasodilator and antithrombotic agent whose effects result from binding to a unique heptahelical G protein-coupled receptor termed the I

prostanoid (IP)4 receptor. This receptor is coupled to $G_s$- and activates adenylate cyclase, resulting in an acute burst of intracellular cAMP. Since expression of CFTR and mutated CFTR is dependent on cAMP-dependent, substances which enhance intracellular levels of cAMP are of interest for development of drugs for treatment of CF. Most of these substances, such as forskolin, however, induce a rather unspecific elevation of cAMP, which may have also very harmful effects such as inflammation. Thus there is an unmet need of specific enhancers of cAMP in lung epithelial cells.

Treprostinil is a potent IP receptor agonist, although its specificity for this receptor is unknown. Sprague R.S. et al., Microcirculation, 2008 Jul;15(5):461-71, showed that Prostacyclin analogues (UT-15, Remodulin) stimulate receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes.

Anderson P. and Graseman H. et al. describe therapeutic methods for treating cystic fibrosis (Anderson, P., Therap. advances in resp. disease, 2010, Vol. 4, No. 3, 177-185; Grasemann H. et al., Exp. Opinion on emerging drugs, 2010, Vol. 15, No. 4, 653-659).

Sprague R. S. et al., describe the stimulatory effect of Iloprost and UT-15C on receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes. Prostacyclin analogs stimulate receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes (Microcirculation 2008, Vol. 15, No. 3, 461-471).

WO2008/104920 A1 discloses the use of quaternary pyridium compounds for the treatment of diseases associated with vascular endothelium dysfunction or liver injury.

EP1712220 A1 describes aerosol compositions comprising poorly water-soluble agents in combination with a phospholipid.

Cycloalkane[bi]indole antagonists of prostaglandin D2 receptors and their use is described in WO2010/008864 A2.

WO2009/049021 A1 describes heterocyclic compounds as CRTH2 receptor antagonists interacting with prostaglandin receptors.

WO2007/071313 A2 describes the use of leukotriene receptor antagonists for the treatment of inflammatory diseases like asthma and COPD.

KR20100086140 describes the use of a leukotriene receptor antagonist for the treatment of CF.

Clinical studies with CF patients treated with Ibuprofen are disclosed by Li J. et al. (Europ. Respiratory Journal, 2008, Vol. 32, No. 2, 334-343).

Ko Shigeru B. H. et al. describe the effect of corticosteroids in the regeneration of cells in autoimmune pancreatitis. (Gastroenterology, 2010, Vol. 138, No. 5, 1988-1996).

WO 02/072108 A1 describes the use of corticosteroids in the treatment of CF.

WO2006/076434 A2 discloses compositions containing a selective COX2 inhibitor and a prostacyclin promotor.

Grasemann H. et al. describe the use of Moli1901 for the treatment of patients with CF (Chest May, 2007, Vol. 131, No. 5, 1461-1466).

A survey on the positive effects of duramycin in the treatment of cystic fibrosis is given by the Committee for Orphan Medicinal Products, "Public Summary of Positive Opinion for Orphan Designation of Duramycin for treatment of cystic fibrosis", European Medicines Agency, 2005

Presently, no treatments of cystic fibrosis are available that significantly improve quality of life of patients over a longer period. Therefore it is an object of the invention to provide compositions for treatment that can enhance the expression of ΔF508 CFTR and/or chloride channel function in epithelial cells of the lung. It is a further object of the invention to provide compositions which can increase the cAMP level in lung cells and which can provide increased levels of cAMP for a longer period of time.

Short description of the invention:

The object of the invention is achieved by providing a composition comprising at least one prostacyclin or prostacyclin analogue or a pharmaceutically acceptable salt thereof and at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin or a pharmaceutically acceptable salt thereof for use in preventing or treating cystic fibrosis.

Specifically, the prostacyclin analogue is selected from the group of Treprostinil, Iloprost, Cicaprost and Beraprost or a pharmaceutically acceptable salt thereof, of acid derivatives of Treprostinil, prodrugs of Treprostinil, polymorphs of Treprostinil or isomers of Treprostinil.

The leukotriene antagonist can be, but is not limited to Montelukast, Zafirlukast, Zileuton and Pranlukast.

The prostaglandin antagonist of the invention can be, but is not limited to the group of laropiprant, NSAIDs, corticosteroids and COX-2 selective inhibitors.

According to an embodiment of the invention the composition is a pharmaceutical composition optionally further containing additional agents known as pharmaceutical additives.

The inventive composition which is used for treatment or prevention of cystic fibrosis can be formulated, but is not limited to, for intravenous administration, inhalation, intravenous or subcutaneous administration, or provided as orally available form selected from the group of sustained release forms, tablets and capsules.

The present invention further provides an *in vitro* method for increasing and optionally stabilizing the cAMP level in a cell wherein said cell is contacted with at least one prostacyclin analogue and at least one compound selected from leukotriene antagonists, prostaglandin antagonists or duramycin.

Alternatively, a therapeutic combination, comprising at least one prostacyclin or prostacyclin analogue and at least one compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists or duramycin, wherein said prostacyclin or prostacyclin analogue and at least one of said compounds are provided in amounts which together are sufficient to treat and/or prevent at least one symptom associated with cystic fibrosis. Specifically, the compounds are formulated for administration by inhalation.

The invention also covers a pharmaceutical combination composition or a kit comprising at least one prostacyclin or prostacyclin analogue and at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin or a pharmaceutically acceptable salt thereof.

Specifically, the inventive composition is formulated as a pharmaceutical composition.

All known administration forms can be used for administering the inventive composition, for example intravenous or subcutaneous administration, or as orally available form selected from the group of sustained release forms, tablets and capsules or by inhalation.

According to a specific embodiment, the effective amount of Treprostinil or a pharmaceutically acceptable salt thereof is preferably at least 1.0 ng/kg of body

weight, the effective amount of Duramycin (MOli1901) preferably is in the range of 0.5 to 5mg, preferably in the range of 0.5 to 2.5mg.

Figures:

Fig. 1: Activation of a Cl-current by Treprostinil in the human bronchial epithelial IB3-1 cell line transiently expressing CFTR-wt.

Detailed description of the invention

It has been surprisingly found by the inventors that prostacyclin or a prostacyclin analogue or a pharmaceutically acceptable salt thereof in combination with at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin may be used in preventing or treating cystic fibrosis. It was shown that a combination of prostacyclin or of a prostacyclin analogue and of at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin may be used to specifically increase the cAMP level in cells, specifically in bronchoepithelial cells from individually suffering from cystic fibrosis (CF) and has thus a synergistic effect in the cellular cAMP increase compared to the use of single substances.

Synthetic prostacyclin analogues can be for example, but are not limited to Treprostinil, Iloprost, Cicaprost or Beraprost.

Treprostinil is marketed as Remodulin™. Treprostinil is a (1*R*,2*R*,3a*S*,9a*S*)-[[2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[f]inden-5-yl] oxy]acetic acid monosodium salt.

Iloprost is marketed as "Ilomedine" and is a 5-{(E)-(1S,5S,6R,7R)-7-hydroxy-6[(E)-(3S, 4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]-bi-cyclo[3.3.0]octan-3-ylidene}pentanoic acid.

Beraprost is a 2,3,3*a*,8*b*-tetrahydro-2-hydroxy-1-(3-hydroxy-4-methyl-1-octen-6-ynyl)-1*H*-cyclopenta(*b*)benzofuran-5-butanoic acid.

Cicaprost is a 2-[(2E)-2-[(3aS,4S,5R,6aS)-5-hydroxy-4-[(3S,4S)-3-hydroxy-4-methylnona-1,6-diynyl]-3,3a,4,5,6,6a-hexahydro-1H-pentalen-2-ylidene] ethoxy]acetic acid.

In reference to prostacyclin, leukotriene antagonist, prostaglandin antagonist and duramycin according to the present invention, the term "prostacyclin analogues", "leukotriene antagonist", "prostaglandin antagonist" and "duramycin" means derivatives, analogues or pharmaceutically acceptable salts of said substances. The

terms "analogue" or "derivative" relate to a chemical molecule that is similar to another chemical substance in structure and function, often differing structurally by a single element or group, which may differ by modification of more than one group (e.g. 2, 3, or 4 groups) if it retains the same function as the parental chemical. Such modifications are routine to skilled persons, and include, for example, additional or substituted chemical moieties, such as esters or amides of an acid, protecting groups such as a benzyl group for an alcohol or thiol, and tert-butoxylcarbonyl groups for an amine. Also included are modifications to alkyl side chains, such as alkyl substitutions (e.g. methyl, dimethyl, ethyl, etc.), modifications to the level of saturation or unsaturation of side chains, and the addition of modified groups such as substituted phenyl and phenoxy. Derivatives can also include conjugates, such as biotin or avidin moieties, enzymes such as horseradish peroxidase and the like, and radio-labeled, bioluminescent, chemoluminescent, or fluorescent moieties. Further, moieties can be added to the agents described herein to alter their pharmacokinetic properties, such as to increase half-life in vivo or *ex vivo*, or to increase their cell penetration properties, among other desirable properties. Also included are prodrugs, which are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.). The term "derivative" also includes within its scope alterations that have been made to a parent sequence including additions, deletions, and/or substitutions that provide for functionally equivalent or functionally improved molecules.

Suitable prostacyclin derivatives include, but are not limited to, acid derivatives, pro-drugs, sustained release forms, inhaled forms and oral forms of Treprostinil, Iloprost, Cicaprost or Beraprost.

According to a specific embodiment of the invention, the Treprostinil derivative is selected from the group of acid derivatives of Treprostinil, prodrugs of Treprostinil, polymorphs of Treprostinil and isomers of Treprostinil.

Similarly, Iloprost, Cicaprost or Beraprost derivatives can be derivatives from the group of acid derivatives, prodrugs, polymorphs or isomers therefrom. The term prostacyclin analogue also covers pharmaceutically acceptable salts thereof.

Specifically, physiologically acceptable salts of prostacyclin analogues include salts derived from bases. Base salts include ammonium salts (such as quaternary ammonium salts), alkali metal salts such as those of sodium and potassium, alkaline earth metal salts such as those of calcium and magnesium, salts with organic bases

such as dicyclohexylamine and *N*-methyl-D-glucamine, and salts with amino acids such as arginine and lysine.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, mangan, potassium, sodium, zinc and the like. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine, caffeine, choline, *N,N'*-dibenzylethylene-diamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, *N*-ethylmorpholine, *N*-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like.

Specifically, Treprostinil is advantageous according to the invention. Treprostinil can successfully enhance the expression of ΔF508 CFTR and/or the chloride channel function in epithelial cells of the lung of cystic fibrosis patients.

It has been surprisingly shown that a prostacyclin analogue in combination with at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin leads to synergistic stimulation of cAMP production and/or increase of cAMP content in bronchoepithelial cells as well as increased stability of the cAMP level in the cells.

Given this ability to stimulate cAMP production through the IP receptor, and the limited presence of IP receptors to a small number of cell-types (such as epithelial lung cells), a prostacyclin or analogue thereof, for example Treprostinil might induce expression and gating of CFTR and mutCFTR in a specific manner which can be used for treatment of CF, in particular, when combined with leukotriene

antagonists, prostaglandin antagonists or duramycin to induce a long lasting increase in cAMP levels within the airway epithelium.

Leukotriene antagonists (referred to as a leukast) are compounds that inhibit leukotrienes, which are fatty signaling molecules produced by the immune system that cause inflammation in asthma and bronchitis, and constrict airways.

Leukotriene inhibitors (or modifiers), such as Montelukast, Zafirlukast, Pranlukast and Zileuton, are used to treat those diseases.

Leukotriene antagonists or leukotriene receptor antagonists – the terms are used interchangeably - of the invention may inhibit or decrease the formation of e.g. leukotrienes $B_4$, $C_4$, $D_4$ or $E_4$ or decreased, or inhibit or attenuate the activation of a Cys-LT receptor (e.g. CyS-$LT_1$ or CyS-$LT_2$). The leukotriene antagonists may also inhibit microsomal glutathione S-transferases (MGSTs), such as MGST-I, MGST-II and/or MGST-III (preferably, MGST-II), thereby inhibiting or decreasing the formation of $LTB_4$ ,$LTD_4$ ,$LTE_4$ or, especially, $LTC_4$. The leukotriene antagonists can also inhibit the activity of 5-lipoxygenase- activating protein (FLAP).

Preferred antagonists are Montelukast, Zafirlukast, Pranlukast and Zileuton.

A preferred leukotriene receptor antagonist according to the invention is Montelukast sodium, which is a selective and orally active LTRA that inhibits the cysteinyl leukotriene CysLT1 receptor. Montelukast sodium is described chemically as [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl]propyl]thio]methyl]cyclopropane acetic acid, monosodium salt.

Montelukast sodium is a hygroscopic, optically active, white to off-white powder. It is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile. It is commercially available. Each 10-mg film-coated Singulair® tablet contains 10.4 mg Montelukast sodium, which is the molar equivalent to 10.0 mg of free acid, and various inactive ingredients. Each 5-mg chewable Singulair® tablet contains 5.2 mg Montelukast sodium, which is the molar equivalent to 5.0 mg of free acid, and various inactive ingredients.

Zafirlukast is a cyclopentyl {3-[2-methoxy-4-({[(2-methylphenyl)sulfonyl]amino} carbonyl)benzyl]-1-methyl-1*H*-indol-5-yl}carbamate. Zafirlukast blocks the action of the cysteinyl leukotrienes on the CysLT1 receptors, thus reducing constriction of the airways, build-up of mucus in the lungs and inflammation of the breathing passages.

Pranlukast is a cysteinyl leukotriene receptor-1 antagonist and is a *N*-[4-oxo-2-(1*H*-tetrazol-5-yl)-4*H*-chromen-7-yl]-4-(4-phenylbutoxy)benzamide.

Zileuton is an orally active inhibitor of 5-lipoxygenase, and thus inhibits leukotrienes ($LTB_4$, $LTC_4$, $LTD_4$, and $LTE_4$) formation and is a (*RS*)-*N*-[1-(1-benzothien-2-yl)ethyl]-*N*-hydroxyurea.

Specifically, the composition for use in preventing or treating CF, specifically by raising the cAMP levels in the bronchoepithelial cells of individuals suffering from CF can specifically comprise Treprostinil and Montelukast or Treprostinil and Zafirlukast or Treprostinil and Pranlukast or Treprostinil and Zileuton.

Alternatively, the composition can specifically comprise Beraprost and Montelukast or Beraprost and Zafirlukast or Beraprost and Pranlukast or Beraprost and Zileuton.

According to a further embodiment, the composition may comprise Cicaprost and Montelukast or Cicaprost and Zafirlukast or Cicaprost and Pranlukast or Cicaprost and Zileuton.

The embodiment of the invention also provides a composition specifically containing Iloprost and Montelukast or Iloprost and Zafirlukast or Iloprost and Pranlukast or Iloprost and Zileuton.

Prostaglandin antagonists are hormone antagonists acting upon prostaglandin.

Examples of known prostaglandin antagonists comprise, but are not limited to Nonsteroidal anti-inflammatory drugs (NSAIDs) which inhibit cyclooxygenase and reduce prostaglandin synthesis or corticosteroids which inhibit phospholipase $A_2$ production by boosting production of lipocortin, an inhibitor protein. Relatively new drugs, known as COX-2 selective inhibitors or coxibs, are used as specific inhibitors of COX-2.

Specifically, the composition for use in preventing or treating CF, specifically by raising the cAMP levels in the bronchoepithelial cells of individuals suffering from CF can specifically comprise Treprostinil and a COX-2 inhibitor or Baraprost and COX-2 inhibitor or Cicaprost and a COX-2 inhibitor or Iloprost and a COX-2 inhibitor

NSAIDs can be but are not limited topropionic acid derivatives like Ibuprofen' Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen; Acetic acid derivatives like Indomethacin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone; Enolic acid (Oxicam) derivatives like Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Isoxicam, Fenamic acid derivatives( Fenamates) like Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, selective

COX-2 inhibitors (Coxibs) like Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib; Sulphonanilides like Nimesulide or Licofelone

According to a further embodiment of the invention NSAIDs can also be selected from, but are not limited to, the group of salicylic acid, acetylsalicylic acid (aspirin), salsalate, bis-salicylate, benzyl-benzoic acid, diflunisal, fendosal, acemetacin, cinmetacin, tolmetin, zomepirac, fenclofenac, isoxepac, benoxaprofen, indoprofen, pirprofen, carprofen, niflumic acid, flunixin, clonixin, phenylbutazone, feprazone, apazone, trimethazone, mofebutazone, kebuzone, suxibuzone.

The second compound in the inventive composition can also be a stimulator of chloride channel ion transport like duramycin (Moli 1901). Duramycin is a stable 19-residue polycyclic peptide that is derived from Streptomyces cinnamoneum. It interacts with phospholipids that are present in plasma and organelle membranes where it activates the chloride channel by elevating intracellular calcium channels (Grasemann et al, 2007, Chest, 131, 1461-1466).

The prostaglandin antagonist can specifically also be Laropiprant which is a (-)-[(3*R*)-4-(4-chlorobenzyl)-7-fluoro-5-(methylsulfonyl)-1,2,3,4-tetrahydrocyclo-penta[b]indol-3-yl]acetic acid and acts as a $DP_1$ antagonist, reducing vasodilation.

According to the invention the term "at least one" or "a" means that one type of prostacyclin or prostacyclin analogue and one type leukotriene antagonists, prostaglandin antagonists and/or duramycin is present for use in the treatment or prevention of cystic fibrosis. However, the composition may also comprise more than one type of prostacyclin or prostacyclin analogue and leukotriene antagonist, prostaglandin antagonist and/or duramycin, specifically two, three, four or more than four types or any combinations of prostacyclins or prostacyclin analogues and leukotriene antagonists, prostaglandin antagonists or duramycin.

According to a preferred embodiment, the composition for use in preventing or treating CF, specifically by raising the cAMP levels in the bronchoepithelial cells can specifically comprise Treprostinil and Moli1901 or Baraprost and Moli1901 or Cicaprost and Moli1901 or Iloprost and Moli1901.

The inventive composition can be formulated as a pharmaceutical composition.

The composition of the invention can be present in any form which can be used for administration.

The specific dose of a compound administered according to this invention to obtain therapeutic or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the route of administration, the age, weight and response of the individual patient, the condition being treated and the severity of the patient's symptoms.

In general, the compounds of the invention are most desirably administered at a concentration that will generally afford effective results without causing any serious side effects and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

The composition can be provided in a variety of systemic and topical formulations. The systemic or topical formulations of the invention are selected from the group of oral, intrabuccal, intrapulmonary, rectal, intrauterine, intradermal, topical, dermal, parenteral, intratumor, intracranial, intrapulmonary, buccal, sublingual, nasal, subcutaneous, intravascular, intrathecal, inhalable, respirable, intraarticular, intracavitary, implantable, transdermal, iontophoretic, intraocular, ophthalmic, vaginal, optical, intravenous, intramuscular, intraglandular, intraorgan, intralymphatic, slow release and enteric coating formulations. The actual preparation and compounding of these different formulations is known in the art and need not be detailed here. The composition may be administered once or several times a day.

Formulations suitable for respiratory, nasal, intrapulmonary, and inhalation administration are preferred, such as topical, oral or parenteral formulations. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into desired formulations.

Compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing the composition as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion.

Compositions suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the active compound, which preparations are preferably isotonic with the blood of the recipient. These preparations may contain anti-oxidants, buffers, bacteriostatic agents and solutes which render the compositions isotonic with the blood of the recipient. Aqueous and non-aqueous

sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried or lyophilized condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

Nasal and instillable formulations comprise purified aqueous solutions of the active compound with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes.

The composition disclosed according to the invention may be administered into the respiratory system either by inhalation, respiration, nasal administration or intrapulmonary instillation (into the lungs) of a subject by any suitable means, and are preferably administered by generating an aerosol or spray comprised of powdered or liquid nasal, intrapulmonary, respirable or inhalable particles. The respirable or inhalable particles comprising the active compound are inhaled by the subject, e.g. by inhalation or by nasal administration or by instillation into the respiratory tract or the lung itself. The formulation may comprise respirable or inhalable liquid or solid particles of the active compound that, in accordance with the present invention, include respirable or inhalable particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and continue into the bronchi and alveoli of the lungs. In general, particles ranging from about 0.05, about 0.1, about 0.5, about 1, about 2 to about 4, about 6, about 8, about 10 microns in diameter. More particularly, about 0.5 to less than about 5 μm in diameter, are respirable or inhalable. Particles of non-respirable size which are included in an aerosol or spray tend to deposit in the throat and be swallowed. The quantity of non-respirable particles in the aerosol is, thus, preferably minimized. For nasal administration or intrapulmonary instillation, a particle size in the range of about 8, about 10, about 20, about 25 to about 35, about 50, about 100, about 150, about 250, about 500 μm in diameter is preferred to ensure retention in the nasal cavity or for instillation and direct deposition into the lung. Liquid formulations may be squirted into the respiratory tract or nose and the lung, particularly when administered to newborns and infants.

Aerosols of liquid particles comprising the active compound may be produced by any suitable means, such as with a nebulizer. Nebulizers are commercially available devices which 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. Suitable compositions for use in nebulizer consist of the active ingredient in liquid carrier, the active ingredient comprising up to 40% w/w composition, but preferably less than 20% w/w carrier being 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 composition is not prepared sterile, for example, methyl hydroxybenzoate, anti-oxidants, flavoring agents, volatile oils, buffering agents and surfactants. Aerosols of solid particles comprising the active compound may likewise be produced with any sold particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicament, product particles which 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. Examples of such aerosol generators include metered dose inhalers and insufflators.

In one embodiment, the delivery device comprises a dry powder inhalator (DPI) that delivers single or multiple doses of the composition. The single dose inhalator may be provided as a disposable kit which is sterilely preloaded with enough formulation for one application. The inhalator may be provided as a pressurized inhalator, and the formulation in a piercable or openable capsule or cartridge. The kit may optionally also comprise in a separate container an agent such as other therapeutic compounds, excipients, surfactants (intended as therapeutic agents as well as formulation ingredients), antioxidants, flavoring and coloring agents, fillers, volatile oils, buffering agents, dispersants, surfactants, antioxidants, flavoring agents, bulking agents, propellants and preservatives, among other suitable additives for the different formulations.

Due to the high metabolic stability of some prostacyclin analogues like Treprostinil, or if provided as lipid based or pegylated forms of the prostacyclins or prostacyclin analogues, the substances can also be administered as depot medicaments.

Most of the leukotriene antagonists, prostaglandin antagonists and duramycin are also metabolically stable, therefore the combination of the prostacyclin analogs and the leukotriene antagonists, prostaglandin antagonists and duramycin can also be formulated as depot medicaments.

Aerosolized delivery of the composition may result in a more homogeneous distribution of the agent in a lung, so that deep lung delivery is obtained. Thereby the dosage of application may be reduced due to the sustained presence of the agent at the site of action in the lung.

The composition can for example be given by nebulizer. The advantage of the nebulizer method of delivery is that less of the substance reaches the systemic circulation. The composition can be given several times a day, for example two or five to ten times a day, however due to the synergistic effect of the prostacyclin analogue and the at least one additional compound selected from leukotriene antagonists, prostaglandin antagonists and duramycin, the dosing frequency may generally be reduced.

The composition can be administered with any pharmaceutically acceptable substances or carriers or excipients as known in the art. These can be for example, but are not restricted to water, neutralizing agents like NaOH, KOH, stabilizers, DMSO, saline, betaine, taurine etc.

The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. The formulation should be selected according to the mode of administration.

The amount of the inventive composition can be selected by any skilled person, specifically the amount of the prostacyclins or prostacyclin analogues or pharmaceutically acceptable salts thereof, specifically of Treprostinil is at least 1.0 ng/kg of body weight. The amount of leukotriene antagonists, prostaglandin antagonists and duramycin can be easily selected by skilled persons, too.

For example, duramycin can be administered in an amount of about 0.5 to 2.5mg. Montelukast can be administered in an amount between 2mg to 200mg.

The present invention additionally provides a method for increasing the cAMP level in a cell wherein said cell is contacted with at least one prostacyclin or prostacyclin analogue and at least one compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin. The increase of cAMP in said cells can be at least 10%, preferably at least 25%, preferably at last 50%, more preferred at least 100% compared to a treatment with prostacyclin, a leukotriene antagonist, prostaglandin antagonist or duramycin alone.

A therapeutic combination, comprising at least one prostacyclin analogue and at least one leukotriene antagonist, prostaglandin antagonist and duramycin, wherein the prostacyclin analogue and at least one compound selected from leukotriene antagonists, prostaglandin antagonists and duramycin are provided in amounts which together are sufficient to treat and/or prevent at least one symptom associated with cystic fibrosis is provided, too. Specifically, at least one of the prostacyclin analogue and at least one agent selected from leukotriene antagonists, prostaglandin antagonists and duramycin are formulated for administration by inhalation.

In a specific embodiment of the present invention, a combination therapy is disclosed for treating cystic fibrosis. In one embodiment, the combination therapy involves administering an effective amount of a prostacyclin or prostacyclin analogue in combination with a compound selected from leukotriene antagonists, prostaglandin antagonists and duramycin. Possibly, one or more additional agents can also be administered.

The prostacyclin or prostacyclin analogue and the compound from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin may be administered together, for example in a single tablet or capsule or inhalation formulation. Alternatively, the compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin and optional additional agents may be administered separately from the prostacyclin or prostacyclin analogue.

The invention further provides a kit and its use for treating or preventing a condition associated with cystic fibrosis in a subject, comprising (i) an effective amount of a prostacyclin or prostacyclin analogue, (ii) a compound from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin (iii) one or more pharmaceutically acceptable carriers and/or additives, and (iv)

instructions for use in treating or preventing cystic fibrosis in a subject, preferably in a human.

Said components (i) and (ii) and (iii) can be in a form suitable for intravenous administration, for inhalation or for oral administration.

The examples described herein are illustrative of the present invention and are not intended to be limitations thereof. Different embodiments of the present invention have been described according to the present invention. Many modifications and variations may be made to the techniques described and illustrated herein without departing from the spirit and scope of the invention. Accordingly, it should be understood that the examples are illustrative only and are not limiting upon the scope of the invention.

Examples

Example 1:

IB3-1 cells are plated on 6 well- plates (0.2*106 cells/well in Fig. 1; 0.4 * $10^6$ cellsin complete growth medium (LHC-8 + 5% FCS). The following day, the adenine nucleotide pool are metabolically labeled by incubation with [3H]adenine (1µCi/well) in Dulbecco's Modified Eagle Medium (DMEM) containing adenosine deaminase (1 unit/ml) for 4h. Thereafter the medium is replaced with fresh medium; the cells are stimulated by sole addition of Treprostinil (in logarithmically spaced concentrations ranging from 0.1 to 30 µM) or of Treprostinil in combination with the indicated concentrations of leukotriene antagonists (e.g., Montelukast, Zafirlukast, Zileuton and Pranlukast, each tested up to 10 µM), prostaglandin antagonists/NSAIDS (Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, each tested at concentrations up to 10 µM) or duramycin (tested up to 30 µM).. After an incubation of 30 min, the cells are lysed by the addition of perchloric acid.

The formation of [3H]cAMP is determined by sequential chromatography on Dowex 50WX-4 and neutral alumina columns followed by liquid scintillation counting of the eluate. The assay is performed in triplicate.

Example 2:

IB3-1 cells endogenously express only mutated CFTR-ΔF508, which is retained within the cells. Using appropriated manipulations (e.g., pharmaco-chaperones or low temperature incubations), it is possible to translocate the mutant CFTR-ΔF508 from the endoplasmic reticulum to the ER; when inserted at the cell

surface, a Cl- conductance can be stimulated by elevating cAMP. The resulting Cl-conductance, however, is small. In order to unequivocally prove that the cAMP accumulation induced by Treprostinil translated into an activation of CFTR, we transiently express a GFP-tagged version of wild type CFTR (the GFP tag allowed for the identification of cells that expressed the protein at the cell surface). As can be seen from Fig. 1, Treprostinil caused a robust activation of the current induced by a depolarization from -40 mV holding potential to + 60 mV. The maximum effect is delayed, i.e. it is only observed several seconds after wash-in of the compound. Likewise, there is also a hysteresis in the turn-off reaction; the current decayed to basal only ~ 100 s after washout. These delayed responses reflect the (i) intervening signaling cascade (i.e., the receptor-dependent activation of $G_s$, $G\alpha_s$-dependent activation of cAMP formation and the ensuing protein kinase A-dependent phosphorylation of CFTR) and (ii) the delayed deactivation of increased cAMP by phosphodiesterases. Similar delays are also seen, if cells are stimulated with forskolin, a direct activator of adenylyl cyclase, which is used as a positive control.

These observations prove that Treprostinil can activate CFTR in bronchial epithelial cells.

Methods:

*Electrophysiology*

The *whole cell patch clamp technique* is used for current recordings performed at 22 ± 1.5°C using an Axoclamp 200B patch clamp amplifier (Axon Instruments). Pipettes have resistances between 1 and 2 MΩ when filled with the recording pipette solution (composition: 110 mM CsCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM $K_2$.ATP, 10 mM Hepes, pH adjusted to 7.2 with CsOH). Voltage-clamp protocols and data acquisition are performed with pclamp 6.0 software (Axon Instruments). Data are low-pass filtered at 2 kHz (-3 dB) and digitized at 10-20 kHz. Cells are continuously superfused with external solution (composition: 145 mM NaCl, 4.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM Hepes, pH adjusted to 7.4 with NaOH). When indicated, the external solution contains Treprostinil (10 µM) or forskolin (5 µM), switching between solutions is achieved by electronically controlled pressure valves.

A sustained response is expected, if Treprostinil is combined with the leukotriene antagonists (Montelukast, Zafirlukast, Zileuton and Pranlukast), prostaglandin antagonists/NSAIDS (Ibuprofen, Naproxen, Fenoprofen, Ketoprofen,

Flurbiprofen, Oxaprozin, Loxoprofen, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib) or duramycin..

*Cell culture:*

I3B-1 cells are grown on dishes (Nunc, 3.5 cm diameter) covered with fibronectin (10 µg/mL) rat collagen I (30 µg/mL) and BSA 10 µg/mL) in LHC-8 medium (Gibco) containing 5% fetal calf serum (FCS). Cells are transiently transfected with a plasmid driving the expression of human GFP-tagged wild type CFTR by using Lipofectamine plus® (Invitrogen) according to the instructions of the manufacturer.

Representative current amplitudes are recorded in the whole cell patch clamp configuration at + 60 mV. A transiently transfected IB3-1 cell expressing GFP-tagged wild type CFTR are selected under fluorescent light and clamped to a holding potential at -40 mV. Depolarization is induced by a voltage step to + 60 mV for 50 ms and the current amplitude was recorded. Wash-in of Treprostinil (10 µM final concentration, TP) is initiated at the time point 50 s and terminated at 125 s. Forskolin is washed in at 275 s and is removed at 375 s. Results are shown in figure 1.

Claims

1. Composition comprising at least one prostacyclin or prostacyclin analogue or a pharmaceutically acceptable salt thereof and at least one additional compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin or a pharmaceutically acceptable salt thereof for use in preventing or treating cystic fibrosis.

2. Composition according to claim 1 wherein said prostacyclin is selected from the group of Treprostinil, Iloprost, Cicaprost and Beraprost or a pharmaceutically acceptable salt thereof.

3. Composition according to claim 1 or 2, wherein said prostacyclin analogue is Treprostinil.

4. Composition according to any one of claims 1 to 3, wherein said prostacyclin analogue is selected from the group of acid derivatives of Treprostinil, prodrugs of Treprostinil, polymorphs of Treprostinil and isomers of Treprostinil.

5. Composition according to any one of claims 1 to 3, wherein said leukotriene antagonist is selected from the group consisting of Montelukast, Zafirlukast, Zileuton and Pranlukast.

6. Composition according to any one of claims 1 to 5, wherein said prostaglandin antagonist is selected from the group consisting of laropiprant, NSAIDs, corticosteroids, COX-2 selective inhibitors.

7. Composition according to any one of claims 1 to 6, wherein said NSAID is selected from the group consisiting of salsalate; propionic acid derivatives, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, loxoprofen; acetic acid derivatives, indomethacin, sulindac, etodolac, ketorolac, diclofenac, nabumetone; enolic acid derivatives, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, isoxicam, fenamic acid derivatives, mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, selective COX-2 inhibitors, celecoxib, rofecoxib, valdecoxib, parecoxib, lumiracoxib, etoricoxib, firocoxib; sulphonanilides, nimesulide, licofelone, salicylic acid, acetylsalicylic acid, bis-salicylate, benzyl-benzoic acid, diflunisal, fendosal, acemetacin, cinmetacin, tolmetin, zomepirac, fenclofenac, isoxepac, benoxaprofen, indoprofen, pirprofen, carprofen, niflumic acid, flunixin, clonixin, phenylbutazone, feprazone, apazone, trimethazone, mofebutazone, kebuzone, suxibuzone.

8. Composition according to any one of claims 1 to 7, wherein said composition is a pharmaceutical composition.

9. Composition according to any one of claims 1 to 8 for intravenous administration.

10. Composition according to any one of claims 1 to 8 for inhalation.

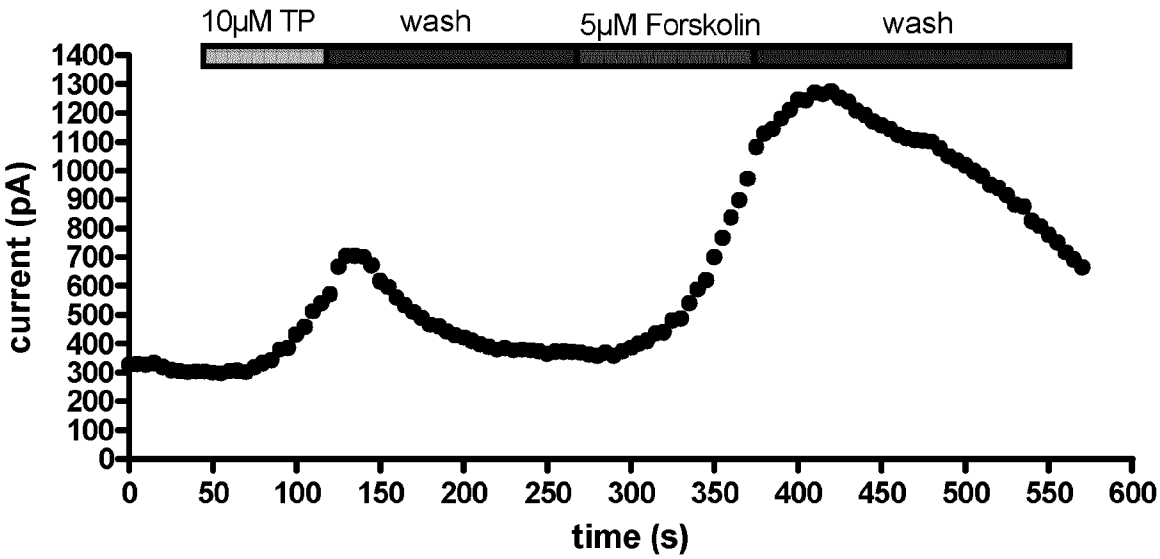
11. Composition according to any one of claims 1 to 8 for subcutaneous administration, or orally available form selected from the group of sustained release forms, tablets and capsules.

12. Method for increasing the cAMP level in a cell wherein said cell is contacted with at least one prostacyclin or prostacyclin analogue and at least one compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists and duramycin or a pharmaceutically acceptable salt thereof.

13. A therapeutic kit, comprising at least one prostacyclin or prostacyclin analogue or a pharmaceutically acceptable salt thereof and at least one compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists or duramycin or a pharmaceutically acceptable salt thereof, wherein said prostacyclin or prostacyclin analogue and at least one of said compounds are provided in amounts which together are sufficient to treat and/or prevent at least one symptom associated with cystic fibrosis.

14. Therapeutic kit according to claim 13, wherein the prostacyclin or prostacyclin analogue or a pharmaceutically acceptable salt thereof and a compound selected from the group consisting of leukotriene antagonists, prostaglandin antagonists or duramycin or a pharmaceutically acceptable salt thereof are formulated for administration by inhalation.

Fig. 1


10µM TP
wash
5µM Forskolin
wash
current (pA)
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
300
350
400
450
500
550
600
time (s)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051881

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K9/28 A61K9/48 A61K31/192 A61K31/343
A61K31/381 A61K31/404 A61K31/41 A61K31/47 A61K38/00
A61K45/06 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ANDERSON PAULA: "Emerging therapies in cystic fibrosis.", THERAPEUTIC ADVANCES IN RESPIRATORY DISEASE, vol. 4, no. 3, June 2010 (2010-06), pages 177-185, XP009147031, ISSN: 1753-4666 the whole document<br>-----<br>GRASEMANN HARTMUT ET AL: "Emerging therapies for cystic fibrosis lung disease.", EXPERT OPINION ON EMERGING DRUGS, vol. 15, no. 4, December 2010 (2010-12), pages 653-659, XP009147065, ISSN: 1744-7623 the whole document<br>-----<br>-/-- | 1-14<br><br>1-14 |

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 February 2012 | 06/03/2012 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Houyvet-Landriscina |

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | TISSIERES P ET AL: "Aerosolized iloprost as a bridge to lung transplantation in a patient with cystic fibrosis and pulmonary hypertension", THE ANNALS OF THORACIC SURGERY, ELSEVIER, vol. 78, no. 3, 1 September 2004 (2004-09-01), pages E48-E50, XP004546014, ISSN: 0003-4975 the whole document | 1-14 |
| Y | SPRAGUE RANDY S ET AL: "Prostacyclin analogs stimulate receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes.", MICROCIRCULATION (NEW YORK, N.Y. : 1994) JUL 2008 LNKD- PUBMED:18574748, vol. 15, no. 5, July 2008 (2008-07), pages 461-471, XP002632351, ISSN: 1549-8719 cited in the application page 461 page 462, column 1, paragraph 3 page 469, column 2, paragraph 2 | 1-14 |
| Y | WO 2008/104920 A1 (TRIGENDO SP Z O O [PL]; GEBICKI JERZY [PL]; MARCINEK ANDRZEJ [PL]; CHL) 4 September 2008 (2008-09-04) page 1, lines 2-12 page 9, line 3 | 1-14 |
| Y | WO 2008/098196 A1 (UNITED THERAPEUTICS CORP [US]; WADE MICHAEL [US]; RICH STUART [US]; SU) 14 August 2008 (2008-08-14) paragraphs [0002], [0006], [0010] - [0011], [0022] - [0023] | 1-14 |
| Y | EP 1 712 220 A1 (PARI GMBH [DE]) 18 October 2006 (2006-10-18) paragraphs [0030], [0103] | 1-14 |
| Y | WO 2010/008864 A2 (AMIRA PHARMACEUTICALS INC [US]; HUTCHINSON JOHN HOWARD [US]; STEARNS B) 21 January 2010 (2010-01-21) paragraphs [0005] - [0006], [0038], [0041], [0047] | 1-14 |
| Y | WO 2009/049021 A1 (CHEMIETEK LLC [US]; YUAN WEI W [US]) 16 April 2009 (2009-04-16) page 1, lines 4-9 page 2, lines 5-9 page 11, lines 15-16 page 16, lines 5-34 | 1-14 |

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2007/071313 A2 (MEDA PHARMA GMBH & CO KG [DE]; MAUS JOACHIM [DE]; KASTRUP HORST [DE];) 28 June 2007 (2007-06-28)<br>page 1, paragraph 1<br>page 2, paragraph 7 - page 3, paragraph 1 | 1-14 |
| Y | DATABASE WPI<br>Week 201079<br>Thomson Scientific, London, GB;<br>AN 2010-K21954<br>XP002632356,<br>& KR 2010 086 140 A (ILDONG PHARM CO LTD)<br>30 July 2010 (2010-07-30)<br>abstract | 1-14 |
| Y | LI J ET AL: "Nonsteroidal anti-inflammatory drugs upregulate function of wild-type and mutant CFTR.", THE EUROPEAN RESPIRATORY JOURNAL : OFFICIAL JOURNAL OF THE EUROPEAN SOCIETY FOR CLINICAL RESPIRATORY PHYSIOLOGY AUG 2008 LNKD- PUBMED:18385167,<br>vol. 32, no. 2, August 2008 (2008-08), pages 334-343, XP002632352,<br>ISSN: 1399-3003<br>abstract<br>page 334<br>page 342, column 1, paragraph 5 - column 2, paragraph 2 | 1-14 |
| Y | KO SHIGERU B H ET AL: "Corticosteroids correct aberrant CFTR localization in the duct and regenerate acinar cells in autoimmune pancreatitis.", GASTROENTEROLOGY,<br>vol. 138, no. 5, May 2010 (2010-05), pages 1988-1996.E3, XP002632353,<br>ISSN: 1528-0012<br>abstract<br>page 1995, column 2, paragraph 2 | 1-14 |
| Y | WO 02/072108 A1 (GLAXO GROUP LTD [GB]; MATTHEWS JOYCE LESLEY [GB]; WEST MICHAEL ROBERT) 19 September 2002 (2002-09-19)<br>page 2, lines 14-23<br>page 3, lines 21-27 | 1-14 |
| Y | WO 2006/076434 A2 (A M TODD COMPANY [US]; FINLEY JOHN WESTCOTT [US])<br>20 July 2006 (2006-07-20)<br>paragraphs [0011] - [0012] | 1-14 |

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | GRASEMANN HARTMUT ET AL: "Inhalation of Moli1901 in patients with cystic fibrosis.", CHEST MAY, vol. 131, no. 5, May 2007 (2007-05), pages 1461-1466, XP002632354, ISSN: 0012-3692 cited in the application abstract<br>----- | 1-14 |
| Y | Committee for Orphan Medicinal Products: "Public Summary of Positive Opinion for Orphan Designation of Duramycin for treatment of cystic fibrosis", European Medecines Agency , 12 December 2005 (2005-12-12), XP002632355, Retrieved from the Internet: URL:http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2009/10/WC500006142.pdf [retrieved on 2011-04-11] the whole document<br>----- | 1-14 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/051881

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 12
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/051881

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2008104920 A1 | 04-09-2008 | CA 2714601 A1 | 04-09-2008 |
| | | EP 2124946 A1 | 02-12-2009 |
| | | RU 2009135821 A | 10-04-2011 |
| | | US 2008221172 A1 | 11-09-2008 |
| | | US 2011118316 A1 | 19-05-2011 |
| | | WO 2008104920 A1 | 04-09-2008 |
| WO 2008098196 A1 | 14-08-2008 | CA 2678258 A1 | 14-08-2008 |
| | | CN 101678033 A | 24-03-2010 |
| | | EP 2120961 A1 | 25-11-2009 |
| | | JP 2010518122 A | 27-05-2010 |
| | | KR 20090117781 A | 12-11-2009 |
| | | US 2008280986 A1 | 13-11-2008 |
| | | WO 2008098196 A1 | 14-08-2008 |
| EP 1712220 A1 | 18-10-2006 | AU 2006233604 A1 | 19-10-2006 |
| | | BR PI0607574 A2 | 15-09-2009 |
| | | CA 2604327 A1 | 19-10-2006 |
| | | EP 1712220 A1 | 18-10-2006 |
| | | EP 1874270 A2 | 09-01-2008 |
| | | WO 2006108556 A2 | 19-10-2006 |
| WO 2010008864 A2 | 21-01-2010 | EP 2300425 A2 | 30-03-2011 |
| | | JP 2011526281 A | 06-10-2011 |
| | | US 2011152338 A1 | 23-06-2011 |
| | | WO 2010008864 A2 | 21-01-2010 |
| WO 2009049021 A1 | 16-04-2009 | AU 2008310874 A1 | 16-04-2009 |
| | | CA 2700824 A1 | 16-04-2009 |
| | | CN 101896178 A | 24-11-2010 |
| | | EP 2197438 A1 | 23-06-2010 |
| | | JP 2011500584 A | 06-01-2011 |
| | | KR 20100065398 A | 16-06-2010 |
| | | RU 2010117156 A | 20-11-2011 |
| | | US 2010222270 A1 | 02-09-2010 |
| | | WO 2009049021 A1 | 16-04-2009 |
| WO 2007071313 A2 | 28-06-2007 | AT 439834 T | 15-09-2009 |
| | | AU 2006329042 A1 | 28-06-2007 |
| | | CA 2632780 A1 | 28-06-2007 |
| | | CN 101321539 A | 10-12-2008 |
| | | DK 1971369 T3 | 16-11-2009 |
| | | EP 1971369 A2 | 24-09-2008 |
| | | EP 2098248 A1 | 09-09-2009 |
| | | ES 2330047 T3 | 03-12-2009 |
| | | HK 1123497 A1 | 21-04-2011 |
| | | HR 20090498 T1 | 31-10-2009 |
| | | JP 2009520711 A | 28-05-2009 |
| | | NZ 568349 A | 27-05-2011 |
| | | PT 1971369 E | 20-10-2009 |
| | | SI 1971369 T1 | 31-12-2009 |
| | | US 2007196285 A1 | 23-08-2007 |
| | | WO 2007071313 A2 | 28-06-2007 |
| KR 2010086140 A | 30-07-2010 | NONE | |
| WO 02072108 A1 | 19-09-2002 | EP 1372666 A1 | 02-01-2004 |
| | | JP 2004533417 A | 04-11-2004 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/051881

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| | | US 2004132700 A1 | 08-07-2004 |
| | | WO 02072108 A1 | 19-09-2002 |
| WO 2006076434 A2 | 20-07-2006 | NONE | |