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(54) **Title:** METHOD FOR TREATING PULMONARY HYPERTENSION

(57) **Abstract:** Pulmonary hypertension (PH) is a progressive syndrome where patients often die of heart failure. The present invention provides a method for treating PH by blocking inflammation. In particular, some aspects of the invention provide a method for treating PH by administering a composition that comprises a compound that is capable of inhibiting or blocking certain types of inflammatory cells or autoimmune responses.



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METHOD FOR TREATING PULMONARY HYPERTENSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 61/750,642, filed January 9, 2013, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a method of treating pulmonary hypertension (PH). In particular, some aspects of the invention provide a method for treating PH by administering a composition that comprises a compound that is capable of inhibiting or blocking certain types of inflammatory cells. In some particular embodiments, the composition comprises a lymphotoxin beta receptor (LTBR) inhibitor, e.g., LTBR-IgG.

BACKGROUND OF THE INVENTION

[0003] Pulmonary hypertension (PH) is a devastating disease that is characterized by progressive increase in pulmonary artery pressure and pulmonary vascular resistance. One of the definitions of PH is a mean pulmonary artery pressure of >25 mmHg at rest or >30 mmHg during exercise with a normal pulmonary capillary wedge pressure, and/or an increased pulmonary vascular resistance index.

[0004] Pulmonary hypertension is a progressive syndrome where the lungs become inflamed and scarred and do not function properly. This inflammation and scarring block important blood vessels entering the lung. When the vessels are blocked, the right side of the heart has to work very hard to keep blood flowing, but eventually PH patients die of heart failure. Currently, there is no effective treatment for reversing or stopping the progression of PH in patients.

[0005] Accordingly, there is a need to develop a method for and/or a composition to prevent and/or reverse pulmonary hypertension.

SUMMARY OF THE INVENTION

[0006] Some aspects of the invention are based at least in part on the discovery by the present inventors that local autoantibody production in pulmonary hypertension (PH) is an important contributing factor to the pathology of PH. Thus, some embodiments of the invention are directed to a method for treating pulmonary hypertension in a subject by administering to a subject in need of such a treatment a composition comprising a

therapeutically effective amount of an auto-antibody antagonist to reduce the activity of auto-antibody produced by bronchus associated lymphoid tissue of said subject. “A therapeutically effective amount” means the amount of a compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will generally vary depending on the compound, the severity of pulmonary hypertension and the age, weight, etc., of the subject to be treated. “Treating” or “treatment” of pulmonary hypertension includes: (1) preventing PH, i.e., causing the clinical symptoms of PH not to develop in a subject that may be exposed to or predisposed to PH but does not yet experience or display symptoms of PH; (2) inhibiting PH, i.e., arresting or reducing the development of PH or its clinical symptoms; or (3) relieving PH, i.e., causing regression of PH or its clinical symptoms. The term “subject” or “patient” refers to any organism to which a composition of this invention can be administered or a method of the invention can be utilized, e.g., for experimental, diagnostic, and/or therapeutic purposes. Typical subjects include animals such as mammals, e.g., mice, rats, rabbits, equines, bovines, felines, canines, non-human primates, and humans.

[0007] Yet in other embodiments, said composition comprises an inhibitor of auto-antibody produced by bronchus associated lymphoid tissue of said subject.

[0008] Still in other embodiments, said composition comprises or can further comprise a compound that reduces the level of pro-inflammatory cytokine in said subject.

[0009] In other embodiments, said composition comprises a compound that reduces immunologic activity of bronchus associated lymphoid tissue.

[0010] Methods of the invention can be used to prevent or reverse pulmonary hypertension in said subject.

[0011] Other aspects of the invention include a method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of a lymphotoxin beta receptor (LTBR) inhibitor to reduce the activity of lymphotoxin beta receptor in bronchus associated lymphoid tissue of said subject. In some embodiments, said LTBR inhibitor comprises LTBR-immunoglobulin (LTBR-Ig).

[0012] Yet other aspects of the invention provide a method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of a lymphotoxin beta receptor (LTBR) gene expression inhibitor. In some embodiments, said composition comprises a LTBR gene transcription inhibitor. Yet in other embodiments, said composition

comprises a LTBR gene translation inhibitor. Still in other embodiments, said LTBR gene expression inhibitor reduces the amount of LTBR gene expression by at least 10% compared to the amount of LTBR gene expression in absence of said LTBR gene expression inhibitor.

[0013] Further aspects of the invention provide a method a method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of a lymphotoxin beta receptor (LTBR) inhibitor. In one particular embodiment, the LTBR inhibitor is LTBR immunoglobulin (i.e., LTBR-Ig).

[0014] Other aspects of the invention include treating PH by administering to the subject in need of such a treatment a vascular endothelial growth factor receptor 3 inhibitor, e.g., MAZ-51.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention is based at least in part on the discovery by the present inventors that local autoantibody production in PH is an important contributing factor to the pathology of PH.

[0016] The present inventors have discovered that *inter alia* both the hypoxia and monocrotaline (MCT)-induced models of PH caused lung injury sufficient to stimulate the rat BALT, thereby resulting in lack of self-tolerance manifested by the local production of autoantibodies. Furthermore, the present inventors have observed that subsequent autoantigenic labeling of vascular cells with autoantibody results from, and is perpetuated by, the inflammation and vascular remodeling typical of PH. It was found that in both the chronic hypoxia and the monocrotaline (MCT) induced rat models of PH, bronchus-associated lymphoid tissue was expanded compared to controls. In addition, rat models of PH had BALT with an activated follicular organization and which were extensively vascularized.

[0017] In MCT treated rats, and to a lesser extent in hypoxic rats, high titers of plasma IgG that labeled lung vascular proteins in a variety of experimental settings were observed. To determine the pathobiological contributions of both BALTs and autoantibodies in PH, pharmacologic, immunologic, and passive transfer strategies in distinct PH models and genetic backgrounds were used. It was found that BALT and autoantibodies were sufficient to cause vascular remodeling and PH.

[0018] The primary histopathological finding in all cases of PH is vascular remodeling, in which the pulmonary vasculature becomes stiff, sometimes occluded, and fibrotic. It is believed that vascular remodeling requires lung infiltration of inflammatory

cells. Continual influx of these inflammatory cells likely sustains a cycle of perpetual inflammation and remodeling by currently not yet understood disease mechanisms. The present invention is based at least in part on the discovery of a mechanism in development of PH.

[0019] The bronchovascular space is a complex structure that has both sterile (vasculature) and non-sterile (airway) components. Bronchus-associated lymphoid tissues (BALT) are tertiary lymphoid organs along the airway that provide an immune surveillance apparatus ensuring both airway and vessel homeostatic patency for matching of ventilation to perfusion. They are histologically similar to lymph nodes containing afferent lymphatic connection, such as B cells, T cells, antigen presenting cells, stroma, and a vascular supply. The chief antigen presenting cells in BALT are dendritic cells (DC) expressing OX-62+ (also known as CD103) and/or CCR7+ that organize around airways as an interconnected trans/sub-epithelial network. DCs scavenge antigens, migrate to CCL19 and CCL21-rich BALT via expression of the cognate receptor CCR7, and orchestrate appropriate immune responses and non-responses (anergy) by both T and B cells. It has been shown that CCR7^{-/-} mice spontaneously develop PH and display BALT rich in B and T cells. This result is believed to be due to the fact that CCR7+ DC are no longer present to suppress lymphocytes via anergy.

[0020] Recently, it has been shown that pulmonary lymphoid neogenesis accompanies idiopathic pulmonary arterial hypertension. However, in that study, it was suggested that pulmonary tertiary lymphoid organs in PH could be directing local immunoglobulin-related immune phenomena.

[0021] PH is believed to be a multi-factorial pathophysiological process. Associations between autoimmune disorders have been recognized for well over 40 years. Antibodies against endothelial cells, fibroblasts, phospholipids, and nuclear antigens have all been described in diseases associated with PH. Furthermore, target antigens in fibroblasts have been described as having contractile effects of IgG binding to vascular smooth muscle cells.

[0022] The present inventors have found that BALT numbers increased in some patients with pulmonary hypertension (PH). BALTs are not normally present in human adults but are in children. When seen in adults, the so-called inducible BALT (iBALT) has been shown to be involved in maintenance of self-tolerance and prevention of autoimmunity. In rats, BALTs are normally present though sparsely compared to rats with lung inflammation and/or infection.

[0023] Some aspects of the invention are based on the present inventors' discovery that differences between BALTs from control lung and from PH lung in animal models, such as rats, offer clues as to the development of iBALT in humans. This discovery was made by the present inventors by quantifying the number and size of BALTs from control rats (i.e., non-PH rats) and from rats with PH. The present inventors have discovered that after 4 weeks of either hypoxia or 4 weeks post-monocrotaline injection, BALTs were significantly more abundant and larger compared to controls. Similar results were found regardless of rat strain (Wistar, Wistar Kyoto, Sprague-Dawley, and Lewis strains), as well as in hypoxic mouse and bovine models of PH. Notably, the increase in number of BALTs that the present inventors observed and their histological appearance closely mirrored the BALT in human idiopathic pulmonary artery hypertension (IPAH). Thus, across species and rat strains, it was discovered that BALTs were associated with PH in a manner consistent with human IPAH.

[0024] The present inventors have also discovered that BALTs in PH lung were organized and were immune-activated. Differences between BALTs in wild type rats compared to PH rats were characterized. The present inventors also investigated the presence of immune cells and looked for expression of immune-modulatory cytokines and chemokines. It was found that PH BALTs contained an abundance of CD3+ T cells, CD45RA+ and CD19+ B cells, and dendritic cells expressing either CCR7, CD11c, and/or OX-62 compared to controls. In PH rat lungs, the dendritic cells were present as a "network" that underpinned the adluminal surface of the associated airway. Experiments showed dendritic cells in control rat lungs sparsely populated the bronchovascular milieu but lacked any appreciable organization. Lymphocytes were organized into segregated clusters of B cells and T cells in PH BALTs but much less so in control BALTs. CD21+ follicular dendritic cells were interspersed among the B cell clusters. In MCT treated rats, but not hypoxic rats, B cells were proliferating as evidenced by Ki-67 co-positivity. No significant amount of antibodies to either CD138 (plasma cells) or activation-induced cytidine deaminase ("AID", an enzyme that is believed to be required for antibody class-switching) that reliably indicated true staining above background was observed. Among the rat groups, no significant differences were observed in the ability of airway M cells to transport nebulized fluorescent microbeads to BALTs, indicating that there were no significant PH-associated changes in trans-airway antigen transport to BALT, i.e., the capacity for BALT to receive airway-derived antigen.

[0025] The present inventors have also discovered that BALTs in PH lung were well-vascularized and selectively adhesive. Experiments were performed to determine if the

BALTS in PH were associated with increased vascularity in a manner structurally consistent with lymph nodes and secondary immune organs. Experiments showed that PH BALTs were more vascularized compared to controls. Slides showed that PH lymphatic vessels expressing aquaporin1 (AQP-1), LYVE-1+, or Podoplanin+ were more clearly evident, as were vascular cell adhesion molecule+ (VCAM) high endothelial venules. AQP-1+ and VCAM were abundantly expressed by HEV endothelium and by cells lining sinuses consistent with previous localizations. Experiments showed that antibodies against PNAd were not significantly able to distinguish BALT structures or cellular constituents above background in rat lungs. To functionally test adhesion differences that would be consistent with the expression differences that were observed, a Stamper-Woodruff assay was performed on rat lung sections using purified rat spleen L-selectin+ T cells labeled with fluorescent dye. An increased binding of L-selectin+ T cells to PH BALTs was observed compared to control BALTs. This binding could be blocked by pre-incubation of the lung tissue with PNAd blocking antibody, and L-selectin⁻ T cells failed to adhere.

[0026] It was also discovered that plasma from PH rats contained anti-fibroblast antibodies produced in BALTs. Tertiary lymphoid tissues have been associated with maintenance of self-tolerance and implicated in autoimmune disorders. The presence of autoantibodies in humans with PH and target antigens has been previously identified. The present inventors have investigated the possibility that PH rat plasma contained autoantibodies similar to those in humans with PH and looked for evidence of autoantibody production in BALT. When plasma from monocrotaline treated rats (12/15) was applied to lung sections from control rats followed by fluorescent-labeled secondary antibody against rat IgG, an intense adventitial staining and small vessel staining was observed. Such staining was absent when the control (i.e., non-hypertensive) rat plasma was applied. Similarly, control rat plasmas applied to lung sections from PH rats, either hypoxic lung or MCT treated, failed to generate any significant staining. Plasmas from a small number of 4-week hypoxic rats (n=3/15) stained control rat lung tissue as well. Both protein G-mediated IgG removal as well as serial dilution of the PH plasmas eliminated the staining, excluding the possibility of background contributions from the secondary antibody or non-specific binding of plasma proteins.

[0027] To further ascertain some specificity as to putative autoantigens, hypertensive rat plasma was incubated with cell lysates of pulmonary artery adventitial fibroblasts that were either mechanically lysed or induced to undergo apoptosis by tunicamycin. Following the incubation and centrifugation, the resulting supernatant failed to stain control lung

sections, indicating that fibroblast lysates contain autoantigens. In order to identify antigen specificity, Alexa-594 conjugated vimentin, phosphatidylinositol 3 kinase (PI3K) or Hsp27 was incubated with rat lung sections. BALTs in PH rat sections, but not controls, co-stained with CD45RA (B cell) and anti-rat IgG antibodies. These results indicate that in situ autoantibody production was ongoing in MCT treated rat BALTs and B cells/plasma cells. Plasma IgG was quantified as PH developed in the MCT treated rats. It was discovered that plasma IgG levels in MCT treated rats increased over controls at 7 days (1120 ± 24 ug/mL), tapered at week 2 (2400 ± 74 ug/mL) and week 3 (1280 ± 68 ug/mL), but then dramatically increased at week 4 (4160 ± 74 ug/mL). To determine the extent of tissue antigenicity, control rat tissue lysates were electrophoresed and immunoblotted with PH rat plasma as the primary sera and anti rat IgG as the secondary. Blotting with pooled (n= 3) control rat plasma produced a single band in all lanes at the size expected for IgG. In contrast, blotting with pooled (n=3 per time point) MCT treated (i.e., administered) rat plasmas produced additional bands in lung, thyroid, heart, and skeletal muscle lysate lanes beginning as early as week 1 post-MCT administration, and continuingly evident in 2 and 3-week MCT treated rat plasmas. Plasma from 4-week MCT treated rats produced banding in a range of sizes in all tissue lysates. This observation is consistent with inter- and intra-epitope autoimmune spreading.

[0028] Experiments were conducted to observe actual autoantibody binding of cellular targets in vitro. Briefly, when rat pulmonary artery adventitial cells were cultured, it was observed that MCT treated rat plasmas and a few hypoxic rat plasmas, but not in control rat plasma, contained antibodies that was predominantly selective to fibroblasts, and to a lesser extent alpha smooth muscle actin expressing pulmonary artery cells and pulmonary artery endothelial cells. Staining patterns varied between individual rat plasmas, ranging from intense nuclear, to cytoplasmic, to perinuclear, or filamentous. As before, either pre-incubation of PH plasma with protein G or serial dilution eliminated staining of vascular cells. Similar results were obtained using human pulmonary artery fibroblasts incubated with IPAH plasmas.

[0029] It was also found that PH plasma anti-fibroblast antibodies stimulated pulmonary adventitial fibroblast to produce pro-adhesive and pro-inflammatory cytokines and chemokines. Binding of autoantibodies is known to induce pro-inflammatory phenotypes in target cells, including fibroblasts. Because autoantibodies were active primarily against pulmonary vascular cells in the MCT treated PH rat plasmas, experiments were conducted to determine the possibility that binding of autoantibodies to their tissue

target (e.g., pulmonary adventitial fibroblasts) would modulate cell function. In both human cell/human plasma and rat cell/rat plasma contexts, it was observed that PH plasmas (12/15 MCT treated rats, 2/12 hypoxic rats) resulted in increased stimulation of pulmonary adventitial fibroblasts to produce both IL-1 β and IL-6, and to express VCAM-1, the production of which led to binding of THP-1 monocytes. This effect was not observed when species mismatched IgG was used, or when anti-VCAM-1 antibody was applied just prior to addition of THP-1 cells. Since autoantibodies have been shown to bind Fc-gamma receptors ("Fc γ Rs"), and since serum amyloid protein ("SAP") binds to all three classical Fc γ Rs, with a preference for Fc γ RI and Fc γ RII, experiments were conducted to test whether SAP could block anti-fibroblast antibodies (AFA) binding and subsequent fibroblast activation. Indeed, SAP in a dose-dependent manner, inhibited the binding of AFA as assessed by immunofluorescence and inhibited the production of IL1 β , IL-6, VCAM-1, and blocked adhesion of THP-1 monocytes.

[0030] More significantly, the present inventors have discovered that reducing or preventing BALT biology prevented and sometimes even reversed PH. To determine functional roles for BALT and autoantibody production in PH, four potential nodes of control were considered: (1) monocrotalinogenic (MCT) treated lung cell apoptosis, (2) CCR7 mediated cell homing, (3) lymphatic vascularization, and (4) BALT formation and maintenance. Salubrinal, a cell stress protectant, was chosen to modulate the unfolded protein response towards a survival phenotype, away from a pro-apoptotic phenotype (control node 1). This experiment was based on discovery by the present inventors that salubrinal protects against MCT-induced broad pneumotoxicity and pulmonary vascular remodeling and PH. When salubrinal was co-administered with MCT and then twice per week thereafter for 4 weeks, a significant reduction of BALT size and number was observed as well as a significant reduction in autoantibody formation in rats. Conversely, salubrinal administered with the same regimen but initiated 2 weeks after MCT treatment did not significantly prevent MCT-induced increases in BALT size, number, or autoantibody development. Surprisingly and unexpectedly, similar results were obtained with the vascular endothelial growth factor receptor 3 inhibitor MAZ-51 (control node 3, lymphatics), which attenuated substantially all endpoints by prevention but less so by reversal regimen. Laser capture microdissection and qPCR of BALT showed a significant decrease in lymphotoxin alpha receptor (LTAR), CXCL13, and activation-induced cytidine deaminase expression compared

to both control and MCT treated rats. It is known that AID is required in class switching recombination [CSR] and somatic hypermutation [SHM] events to occur in B cell selection.

[0031] Antagonism of CCR7 by CCR7-Ig potentiated MCT-PH, BALT, and plasma IgG, with lung remodeling similar to the CCR7^{-/-} mouse phenotype (control node 2). CCR7 antagonism in MCT treated rats is believed to be associated with increased AID, decreased CCR7, CCL19, CCL21, lymphotoxin beta receptor (LTBR), and CXCL13 in BALT.

Surprisingly and unexpectedly, the present inventors have found that inhibition of LTBR (control node 4), e.g., by administering lymphotoxin beta receptor immunoglobulin (LTBR-Ig), resulted in prevention and reversal of MCT-induced PH and vascular remodeling.

Furthermore, BALT were almost completely absent in LTBR-Ig treated MCT rats, and no significant amount of plasma autoantibodies were detected. Table below summarizes results from pharmacologic and immunologic interventions in MCT rats.

Treatment Type ↓	Increased BALT size & BALT # Prevent/Reverse	mean PA pressure (mmHg) Prevent/Reverse	Pulm Vasc Remodeling Prevent/Reverse	Autoabs Prevent/Reverse
CCR7 Antagonism n=5/group	Yes/Yes	83/82	++/+++	√/√
LTbR Antagonism n=5/group	No/No	15/18	-/-	-/-
Passive Antibody Transfer n=3/group	Yes	42	++	√
Unfolded protein response modulation (Salubrinal, cell death protectant)	No/Yes	28/40	-/+	-/√
VEGFR3 Antagonism (MAZ51, lymphangiogenesis inhibitor)	No/Yes	28/45	-/+	-/√

[0032] In addition, it was found that immunization of rats with vascular cell antigens or passive transfer of autoantibodies caused pulmonary vascular remodeling. To determine whether autoimmune-related pathologies directly contributed to the development of PH, two distinct “gain of function” experiments were performed. Briefly, rats were immunized with an apoptotic (induced by tunicamycin) pulmonary artery cell lysate in adjuvant twice per week for 4 weeks. Alternatively, autoantibody-containing plasmas from MCT treated rats were injected into naive rats. Compared to injection of adjuvant alone, adjuvant mixed with intact vascular cells, or pre-immune plasmas, injection of either pulmonary vascular cell apoptotic lysates or autoab⁺ plasmas induced BALT, bronchovascular and resistance vessel remodeling that led to moderately severe PH with right and left ventricular hypertrophy and

fibrosis. Notably, high titers of plasma IgG was found in immunized PH rats. This plasma IgG reacted with lung vascular cells in culture and in tissue sections.

[0033] As disclosed herein, bronchus associated lymphoid tissue in the setting of PH displayed unique cellular consistency, vascular architecture, and genomic programming permissive for production of autoantibodies. The present inventors have also discovered that in rats with either hypoxic or monocrotaline-induced PH, BALTs, which increased in size and number, vascularized in a manner that facilitates L-selectin+ T cell binding and were a source for the local production of activating anti-fibroblast antibodies. It was also discovered that the intracellular proteins vimentin and Hsp27, as well as PI3K, were potential autoantigens in rats with MCT-induced PH. In addition, it was discovered that autoantibodies induced inflammatory phenotypes in pulmonary adventitial fibroblasts, which is believed to be involved in binding of FcγRs. Furthermore, it was shown that the signaling networks of CCR7, VEGFR3, LTBR, and the unfolded protein response were all impacted by the biology of BALT, autoantibodies and PH. These observations confirm the discovery by the present inventors that lung vascular cells are important sources of autoantigens in the monocrotaline treated rat model of PH, and that BALTs are one of the principal sites of disease-causing autoantibody production.

[0034] As noted above, currently there is no cure for pulmonary hypertension despite the decades of advancements in understanding its pathobiology. As disclosed herein, in contrast to the current understanding of PH, some PH results from inflammation and/or vascular remodeling. Accordingly, some aspects of the invention are based on the discovery by the present inventors that locally produced autoantibodies against lung vascular antigens cause pulmonary vascular remodeling and PH. In particular, in some aspects of the invention, a method for treating (i.e., reducing, preventing and/or reversing) PH in a subject is provided. In one particular embodiment, the method of the invention comprises administering a composition comprising a compound that can reduce or inhibit formation or production of such autoantibodies or the activity of such autoantibodies.

[0035] In humans, ectopic lymphoid tissues form in response to local infection or sterile inflammation. Recirculating lymphocytes enter lymphoid tissues via high endothelial venules and leave via lymphatics. Encounters between dendritic cells, macrophages, and other antigen presenting cells with T and B lymphocytes in ectopic lymphoid tissues are believed to facilitate anergy, maintenance of self-tolerance, and mobilization of appropriate adaptive immune responses. It has been shown that BALT develops *inter alia* following

sustained signaling of interleukin (IL)-6 via adenoviral delivery to the lung, in the absence of CCR7, and with the requirement of IL-17.

[0036] As the present disclosure shows, the BALT in MCT treated rats, and to a lesser extent in hypoxic rats, are lymphoid follicles and not lymphocytic aggregates due to lymphocyte segregation, the presence of HEV, the presence of follicular dendritic cells, gene expression repertoire, or antibody class switching. These characteristics were absent from lymphoid follicles in control rat lungs, which were also smaller and less numerous. In both MCT treated and hypoxia PH models, and across genetic backgrounds, the inflammation was sterile. In several models, sterile inflammation was capable of inducing lymphoid neogenesis and autoimmunity. It is believed that both the duration and extent of lung injury are some of the factors that influence BALT development and loss of tolerance.

[0037] MCT is a broad pneumotoxin that causes not only endothelial cell apoptosis, but also alterations in airway cytokine flux, airway constriction and decreased dynamic respiratory compliance. The present inventors have discovered that MCT causes a robust activation of the unfolded protein response (UPR) and CHOP-mediated apoptosis that was most pronounced in bronchial epithelium within 48 hours of MCT administration. These findings, along with the results disclosed herein show that *inter alia* (1) modulating the UPR prevents BALT activation and the production of autoantibodies, and (2) injection of apoptotic cells in adjuvant causes PH. Brefeldin A, tunicamycin, and thapsigargin are examples of agents which can induce the UPR. Salubrinal, trimethylamine N-oxide, 4-phenylbutyrate (PBA), and tauroursodeoxycholic acid (TUDCA) are example of chemical chaperones that can attenuate the UPR.

[0038] In PH rats compared to control, BALT were larger, more abundant, and more organized and vascularized. Without being bound by any theory, it is believed that one of the factors for this result is a response to increased sampling load of self-antigens following lung injury and thus a requirement for more interactions between DC and lymphocytes, particularly in the context of anergy. The data disclosed herein show that CCR7 is likely a required chemokine for DC egress from the lung parenchyma to lymphoid tissues, similar to its roles in gut inflammation. In fact, localized gene expression and cytokines that are supportive of lymphoid neogenesis were observed. Blockade of LTBR impacts several aspects of BALT neogenesis and maintenance. As disclosed herein, BALT is required for autoantibody generation and PH induced by MCT treatment. Intervention with LTBR-Ig at 14 days post-MCT treatment resulted in no significant establishment of PH. In contrast, autoantibodies were observed within the first week post-MCT treatment in the absence of

LTBR-Ig administration and the average pulmonary artery pressures at 2 weeks were about 35 mm Hg, indicating PH.

[0039] Without being bound by any theory, it is believed that once DC have begun to clear apoptotic lung cells, they must interact with immune cells using co-stimulatory molecules displayed on the reciprocal cell surfaces. It has recently been shown by others that PH in athymic rats was prevented by immune reconstitution with FoxP3⁺ Tregulatory cells (Tregs).

[0040] As shown herein, CCR7 blockade likely inhibits the DC's ability to coordinate an appropriate Treg response. One of the possible explanations for this observation is that subpopulations of Tregs exist (possibly even FoxP3 negative or low) that are CCR7⁺ and were prevented from migrating properly to lymphoid tissues and thus not engaging in anergistic interactions with DC. Tregs and TH17 cells may play a role in modulating the activity of self-reactive B cells. B cells can be activated by both innate (toll-like receptors) and acquired immune mechanisms, and can reciprocally influence T cells and DC. Myeloid derived suppressor cells (MDSC) may also play a role in the autoimmune pathogenesis of PH. The present inventors have observed increased numbers of circulating Myeloid-derived Suppressor Cells (MDSC) in patients with PH, and similar phenotypic cells (DC-SIGN or CD11b⁺/MHCII⁻) localize to remodeled pulmonary vasculature in both human and animal models of PH. The task of tracing and sorting out the functionally cooperative roles of subsets of regulatory myeloid and lymphocytic cells in the development of PH is also possible using the models and approaches disclosed herein.

[0041] As disclosed herein pulmonary vascular remodeling can be induced along with development of PH and cardiac fibrosis in naive rats by injecting them with MCT-derived plasma autoantibodies. Using the 4-week MCT treated rats as the plasma source explains differences in PH between MCT treated rats and rats given autoantibodies (i.e., "autoabs") from MCT treated rats. One of the most striking differences was the extent of the fibrosis in the left ventricle of the autoab recipient rats, which can be attributed to epitope spreading. Another possible explanation for the results is that rats 4 weeks post-MCT treatment were undergoing multi-organ failure and may have been producing autoantibodies accordingly.

[0042] Other aspects of the invention are based on the discovery by the present inventors that autoantibodies in PH can be pathological. In particular, some aspects of the invention provide methods for treating PH by administering a composition comprising a compound that inhibit or reduce the activity of such autoantibodies. It should be appreciated that inhibiting or reducing the activity of autoantibodies can include inhibiting or reducing

the activity of the autoantibody itself, inhibiting or reducing the production (e.g., translation and/or transcription) of the autoantibody, e.g., by using an appropriate siRNA or other similar compounds that can be produced or known to one skilled in the art having read the present disclosure.

[0043] Some aspects of the invention utilize a LTBR antibody, e.g., LTBR-Ig, to treat PH. Isolated antibodies of the invention can include serum containing such antibodies, or antibodies that have been purified to varying degrees. Whole antibodies of the invention can be polyclonal or monoclonal. Alternatively, functional equivalents of whole antibodies, such as antigen binding fragments in which one or more antibody domains are truncated or absent (e.g., Fv, Fab, Fab', or F(ab)₂ fragments), as well as genetically-engineered antibodies or antigen binding fragments thereof, including single chain antibodies or antibodies that can bind to more than one epitope (e.g., bi-specific antibodies), or antibodies that can bind to one or more different antigens (e.g., bi- or multi-specific antibodies), can also be employed in the invention.

[0044] Generally, in the production of an antibody, a suitable experimental animal, such as, for example, but not limited to, a rabbit, a sheep, a hamster, a guinea pig, a mouse, a rat, or a chicken, is exposed to an antigen against which an antibody is desired, e.g., LTBR. Typically, an animal is immunized with an effective amount of antigen (e.g., LTBR) that is injected into the animal. An effective amount of antigen refers to an amount needed to induce antibody production by the animal. The animal's immune system is then allowed to respond over a pre-determined period of time. The immunization process can be repeated until the immune system is found to be producing antibodies to the antigen. In order to obtain polyclonal antibodies specific for the antigen, serum is collected from the animal that contains the desired antibodies (or in the case of a chicken, antibody can be collected from the eggs). Such serum is useful as a reagent. Polyclonal antibodies can be further purified from the serum (or eggs) by, for example, treating the serum with ammonium sulfate.

[0045] Monoclonal antibodies can be produced according to the methodology of Kohler and Milstein (*Nature*, **1975**, 256, 495-497). For example, B lymphocytes are recovered from the spleen (or any suitable tissue) of an immunized animal and then fused with myeloma cells to obtain a population of hybridoma cells capable of continual growth in suitable culture medium. Hybridomas producing the desired antibody are selected by testing the ability of the antibody produced by the hybridoma to bind to the desired antigen.

[0046] Some aspects of the invention provide a composition comprising a LTBR gene expression inhibitor and/or LTBR inhibitor. The term "LTBR gene expression inhibitor"

refers to a composition and/or a compound that reduces translation or transcription of LTBR gene by at least 10%, typically by at least 25%, often by at least 40%, and more often by at least 50%, compared to the amount of LTBR gene expression in the absence of the LTBR gene expression inhibitor. The term "LTBR inhibitor" refers to a composition and/or compound that reduces the activity of LTBR by at least 10%, typically by at least 25%, often by at least 40%, and more often by at least 50%, compared to the amount of LTBR activity in the absence of the LTBR inhibitor. Such inhibition can be readily determined by using the methods known to one skilled in the art having read the present disclosure.

[0047] The compositions of the invention can include an individual isomer, racemic or non-racemic mixture of isomers or a pharmaceutically acceptable salt or solvate thereof of the LTBR gene expression inhibitor or LTBR inhibitor, together with at least one pharmaceutically acceptable carrier, and optionally other therapeutic and/or prophylactic ingredients.

[0048] In general, the compositions of the invention are administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. Suitable dosage ranges are typically 1-500 mg daily, typically 1-100 mg daily, and often 1-30 mg daily, depending on numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, the indication towards which the administration is directed, and the preferences and experience of the medical practitioner involved. One of ordinary skill in the art of treating such diseases is typically able, without undue experimentation and in reliance upon personal knowledge and the disclosure of this application, to ascertain a therapeutically effective amount of the compounds of the invention.

[0049] Typically, compounds of the invention are administered as pharmaceutical formulations including those suitable for oral (including buccal and sub-lingual), nasal, topical, pulmonary, or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. Typical manner of administration is generally oral using a convenient daily dosage regimen which can be adjusted according to the degree of affliction.

[0050] The compositions of the invention, together with one or more conventional adjuvants, carriers, or diluents, can be placed into the form of pharmaceutical compositions and unit dosages. The pharmaceutical compositions and unit dosage forms can be comprised of conventional ingredients in conventional proportions, with or without additional active

compounds or principles, and the unit dosage forms can contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. The pharmaceutical compositions can be employed as solids, such as tablets or filled capsules, semisolids, powders, sustained release formulations, or liquids such as solutions, suspensions, emulsions, elixirs, or filled capsules for oral use; or in the form of sterile injectable solutions for parenteral use. Formulations containing about one (1) milligram of active ingredient or, more broadly, about 0.01 to about one hundred (100) milligrams, per tablet, are accordingly suitable representative unit dosage forms.

[0051] The compositions of the invention can be formulated in a wide variety of oral administration dosage forms. The pharmaceutical compositions and dosage forms can comprise a pharmaceutically acceptable carrier which can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, and dispersible granules. A solid carrier can be one or more substances which can also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier generally is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatine, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term “preparation” is intended to include the formulation of the active compound with encapsulating material as carrier, providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

[0052] Other forms suitable for oral administration include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions can be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents, for example, such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizers, and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in

water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents. Solid form preparations include solutions, suspensions, and emulsions, and can contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

[0053] The compositions of the invention can also be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and can be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and can contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water.

[0054] The compositions of the invention can be formulated for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example, with a dropper, pipette or spray. The formulations can be provided in a single or multidose form. In the latter case of a dropper or pipette, this can be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this can be achieved for example by means of a metering atomizing spray pump.

[0055] The compositions of the invention can be formulated for aerosol administration, particularly to the respiratory tract and including intranasal administration. The compositions will generally have a small particle size for example of the order of five (5) microns or less. Such a particle size can be obtained by means known in the art, for example by micronization. The active ingredient is provided in a pressurized pack with a suitable propellant such as a chlorofluorocarbon (CFC), for example, dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, or carbon dioxide or other suitable gas. The aerosol can conveniently also contain a surfactant such as lecithin. The dose of drug can be controlled by a metered valve. Alternatively the active ingredients can be provided in a form of a dry powder, for example, a powder mix of the compound in a suitable powder base

such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). The powder carrier typically forms a gel in the nasal cavity. The powder composition can be presented in unit dose form, for example, in capsules or cartridges of e.g., gelatine or blister packs from which the powder can be administered by means of an inhaler.

[0056] When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient. For example, the compounds of the invention can be formulated in transdermal or subcutaneous drug delivery devices. These delivery systems are advantageous when sustained release of the compound is necessary or desired and when patient compliance with a treatment regimen is crucial. Compounds in transdermal delivery systems are frequently attached to a skin-adhesive solid support. The compound of interest can also be combined with a penetration enhancer, e.g., Azone (1-dodecylazacycloheptan-2-one). Sustained release delivery systems can be inserted subcutaneously into the subdermal layer by surgery or injection. The subdermal implants encapsulate the compound in a lipid soluble membrane, e.g., silicone rubber, or a biodegradable polymer, e.g., polylactic acid.

[0057] The pharmaceutical preparations are typically in unit dosage forms. In such form, the preparation is often subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

[0058] Other suitable pharmaceutical carriers and their formulations are described in Remington: *The Science and Practice of Pharmacy* 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa.

[0059] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting. In the Examples, procedures that are constructively reduced to practice are described in the present tense, and procedures that have been carried out in the laboratory are set forth in the past tense.

EXAMPLES

[0060] LTBR-Ig and MOPC-21 were obtained from Biogen Idec.

[0061] Rat Models of PH: The characterization of the monocrotaline (MCT) treated rat model has been previously summarized. See, for example, Stenmark et al. in *American*

journal of physiology. Lung cellular and molecular physiology, **2009**, 297, L1013-1032.

Briefly, male Wistar rats were utilized with a single subcutaneous injection of 60 mg/kg at six to eight weeks of age. Lung parenchyma from animals was obtained as previously described by Yeager et al. in *American journal of respiratory cell and molecular biology*, **2012**, 46, 14-22 and *Chest*, **2012**, 141, 944-952. Hemodynamics were assessed with a Millar catheter placed in the main pulmonary artery via the right ventricle; correct placement of the catheter was confirmed by observing a significant rise in diastolic pressure as the catheter moved out of the ventricle. Systemic blood pressure was monitored with another pressure catheter inserted in the femoral artery. Cardiac performance was assessed with a pressure-volume system (Scisense Inc, London, Ontario, Canada). For analyses, animals were anesthetized with 2% isoflurane, and their body temperature was maintained at 37 °C. Total pulmonary vascular resistance index was calculated as mean pulmonary artery pressure (PAP)/cardiac index, where cardiac index equals cardiac output divided by body weight.

[0062] BALT Immunofluorescence & Quantification: Rat tissues were sectioned at 5 µm for either hematoxylin and eosin staining (Sigma), trichrome (Sigma), or immunohistological staining. Antibody isotype negative controls were included with each sample group. Images were acquired at room temperature using a Zeiss Axiovert S100 fitted with Zeiss 20 X 0.4 numerical aperture (n.a.) and 10 X 0.3 n.a. objectives and AxioCam camera. Acquisition of images was by Axiovision 4.6 software (Zeiss).

[0063] Laser Capture Microdissection & qPCR: Veritas Microdissection LCM instrument was used to selectively dissect lymphoid tissue (Arcturus Engineering, Mountain View, CA). Tissue cryosections (20 µm) were applied to PEN membrane slides (Arcturus Engineering), fixed in 70% ethanol (15 s at -20 °C) followed by 100% acetone (5 min at -20 °C), dehydrated in graded ethanol (30 s each) and xylene (10 min), and air-dried. Lymphoid tissue from a given lung section was dissected and placed on a separate region of the same cap. Samples from individual animals were collected and analyzed separately to allow for statistical analysis. Captured tissues were incubated in lysis buffer for 30 min at 42 °C, and total RNA was extracted with the Qiagen RNeasy Micro Kit with DNase treatment (Qiagen, Valencia, CA). RNA was analyzed with the Agilent 2100 Bioanalyzer Pico Chip (Agilent Technologies, Palo Alto, CA). Only RNA samples with clearly defined 18S and 28S rRNA peaks were used for further analysis. The primers used were obtained from Applied Biosystems Inc., with the following product numbers: CCR7 Rn02758813_s1, CCL21 Rn01764651_g1, CCL19 Rn01439563_m1, lymphotoxin alpha receptor

Rn03993492_g1, lymphotoxin beta receptor Rn01754146_g1, CXCL13 Rn01450028_m1, proliferating cell nuclear antigen Rn00574296_g1, AID Rn01492306_m1.

[0064] RNA Amplification and cDNA Synthesis: Amplified cDNA from LCM samples was generated from a minimum of 500 pg of RNA using the WT-Ovation Pico RNA Amplification System (NuGEN Technologies, San Carlos, CA). cDNA yield was determined by measuring A260 on the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed using Taqman primers according to manufacturer's instructions (Applied Biosystems/Perkin Elmer).

[0065] Autoantibody Purification: Rat plasmas were isolated from whole blood by centrifugation in heparinized tubes at room temperature. Single use aliquots were prepared and stored at -80 °C until use. IgG were purified just prior to use via Melon IgG purification kit (Thermo Pierce) per manufacturer's instructions.

[0066] Immunoblot: Immunoblots were performed as previously described by Strassheim et al., 2009 in *J. Immunol.*, **2009** Dec 1; *183(11)*, 6981-8. PMID: 19915063. For autoantibody blots, rat plasma and/or purified were used as the primary antibody at 1:500 dilution.

[0067] ELISAs: Rat plasmas and cell culture supernatants were prepared by centrifugation. The concentrations of IgG, Chemokine (C-C motif) ligand 2 (CCL2), and Interleukin (IL)-6 were then measured according to the manufacturer's instructions (Genway, Novus Biological, and eBioscience, respectively).

[0068] Stamper-Woodruff Adhesion Assay: L-selectin⁺ T lymphocytes were isolated from rat spleens using immunomagnetic beads (Invitrogen, Grand Island, NY) and a rat-specific L-selectin antibody (Novus Biological NB100-63968). PKH-26 dye (Sigma) labeling and subsequent adhesion was performed as previously described by Yeager et al. in *American journal of respiratory cell and molecular biology*, **2012**, *46*, 14-22. In some experiments, peripheral node addressin (PNAd) antibody (Novus Biological) in phosphate buffered saline/0.1% bovine serum albumin was applied for 60 minutes at room temperature just prior to adhesion of T cells.

[0069] Passive Autoantibody Transfer: IgGs were purified from 4 week MCT treated rat plasmas as described above. Alternatively, unfractionated plasma was used in separate experiments. Autoantibodies were injected at 1mg/mL via tail vein injection twice per week for 3 weeks. Prior to any injections, pre-immune plasma was centrifuged from 1 mL peripheral blood drawn from the tail vein that was collected once per week for 2 weeks.

[0070] Pulmonary artery cell immunizations: Pulmonary artery cells were collected as previously described in *American journal of respiratory cell and molecular biology*, **2012**, 46, 14-22. Whole cells, pre-immune plasma, or cells induced to apoptosis by tunicamycin (1 ng/mL, 6 hours), were mixed with TITERMAX[®] per manufacturer's instructions. Cells and TITERMAX[®] were injected twice weekly for 3 weeks.

[0071] In vivo Test and Results: Rats with established PH, via single injection of MCT, was used in this experiment. Without LTBR-Ig treatment these rats will die of right heart failure with 100% certainty by four weeks. For this in vivo test, rats were treated with LTBR-Ig. Tests showed that LTBR-Ig treated PH rats had diminution of lymphoid tissue and a significant and dramatic reduction in pulmonary artery pressure. More significantly, these rats survived into 4 weeks, after which they were sacrificed to conduct experimental observations and measurements.

[0072] A second set of rats with established lethal PH, via Sugen 5416 vascular endothelial growth factor receptor 2 blockade plus 4 weeks of hypobaric hypoxia, were also treated with LTBR-Ig. These rats were similarly protected from PH. More significantly, in rats with established PH, some actually showed reversal to normal pulmonary artery pressures.

[0073] As shown in these experiments, LTBR-Ig, compared to MOPC (IgG control) protected and in some cases actually reversed PH in 2 lethal/severe models of PH that are associated with right ventricle (RV) failure as well as in rat models of moderate PH that is not associated with PH (hypobaric hypoxia alone for 4 weeks). Furthermore, LTBR-Ig was well tolerated in control rats without PH.

[0074] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

What is Claimed is:

1. A method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of an auto-antibody antagonist to reduce the activity of auto-antibody produced by bronchus associated lymphoid tissue of said subject.
2. The method of Claim 1, wherein said composition comprises an inhibitor of auto-antibody produced by bronchus associated lymphoid tissue of said subject.
3. The method of Claim 1, wherein said composition comprises a compound that reduces the level of pro-inflammatory cytokine in said subject.
4. The method of Claim 1, wherein said composition comprises a compound that reduces immunologic activity of bronchus associated lymphoid tissue.
5. The method of Claim 1, wherein said treatment reverses pulmonary hypertension.
6. A method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of a lymphotoxin beta receptor (LTBR) inhibitor to reduce the activity of lymphotoxin beta receptor in bronchus associated lymphoid tissue of said subject.
7. The method of Claim 6, wherein said LTBR inhibitor comprises LTBR-immunoglobulin (LTBR-Ig).
8. A method for treating pulmonary hypertension in a subject, said method comprising administering to a subject in need of such a treatment a composition comprising a therapeutically effective amount of a lymphotoxin beta receptor (LTBR) gene expression inhibitor.
9. The method of Claim 8, wherein said composition comprises a LTBR gene transcription inhibitor.
10. The method of Claim 8, wherein said composition comprises a LTBR gene translation inhibitor.

11. The method of Claim 8, wherein said LTBR gene expression inhibitor reduces the amount of LTBR gene expression by at least 10% compared to the amount of LTBR gene expression in absence of said LTBR gene expression inhibitor.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/010851

A. CLASSIFICATION OF SUBJECT MATTER			<i>A61K 39/395 (2006.01)</i> <i>A61P 11/00 (2006.01)</i>
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 39/395, A61P 11/00			
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 practicable, search terms used)			
Espacenet, RUPTO, USPTO, EAPATIS, PubMed, Google, Patsearch (RUPTO internal)			
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
Y	KURYATA A.V. "Legochhnaya gipertenziya v klinicheskoy praktike" Meditsinskaya gazeta "Zdorovie Ukrainy", July 2003, №74, [online] [retrieved on 21.02.2014] Retrieved from the Internet: <URL:http://health-ua.com/articles/282.html>, p. 1, line 27, p. 3, paragraph 4	1-11	
Y	US 20120251562 A1 (PIERRE PHILIPPE) 04.10.2012, abstract, claims 21, 26, paragraphs [0008], [0013], [0024], [0030]	1-5	
Y	US 20120003235 A1 (BIOGEN IDEC MA INC.) 05.01.2012, paragraphs [0199], [0201], [0202], [0136] - [0138], [0354], [0359], [0455]	2, 4, 6-11	
A	US 20120014947 A1 (THE UNIVERSITY OF CHICAGO) 19.01.2012, claims, paragraphs [0017], [0030]	1-11	
☐ Further documents are listed in the continuation of Box C. ☐ 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	"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
"E"	earlier document but published on or after the international filing date	"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
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"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 28 February 2014 (28.02.2014)		Date of mailing of the international search report 07 May 2014 (07.05.2014)	
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37		Authorized officer N. Litvinenko Telephone No. (495)531-64-81	