Drugs of the Future 2010, 35(11): 947-954

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.11.1556470





# THERAPEUTIC TARGETS FOR PULMONARY HYPERTENSION

*L.A. Sorbera, S. Rajendran, C. Dulsat, N. Forns and E. Rosa*

Thomson Reuters, Barcelona, Spain

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## SUMMARY

*Pulmonary hypertension is a rare, life-threatening, progressive lung disorder characterized by elevated pulmonary arterial pressure and secondary right ventricular failure due to vascular remodeling, plexiform vascular lesions, vasoconstriction and/or thrombosis. There is no cure for pulmonary hypertension, and until recently, the disorder has been neglected by the medical community and many questions remain regarding its etiology. However, renewed interest in pulmonary hypertension has been generated. The ideal therapeutic strategy for pulmonary hypertension is complex, requiring a reduction in pulmonary vascular resistance, an improvement in systemic circulation and an increase in right ventricular inotropy. To date, pulmonary vasodilators, anticoagulants and diuretics are available and combination therapy is a possibility for patients. However, the search continues for more effective treatment strategies for pulmonary hypertension, with investigation focusing on identifying novel targets for therapeutic intervention. This article presents those drug targets that are currently under active investigation for the treatment of pulmonary hypertension.*

## INTRODUCTION

Pulmonary hypertension is a rare, life-threatening, progressive lung disorder characterized by elevated pulmonary arterial pressure above the normal range and secondary right ventricular failure. Symptoms are often nonspecific and may include fatigue after minimal exertion, edema, dizzy spells, anginal chest pain and fainting. The elevated pulmonary pressure may eventually contribute to right ventricular failure and death. The prevalence of pulmonary hypertension is unknown, although it is relatively rare, occurring in approximately 1-2 patients per million population. In the U.S., some 300 new cases are diagnosed each year (1-5).

**Correspondence:** Lisa Ann Sorbera, PhD, Thomson Reuters, Provença 388, 08025 Barcelona, Spain. E-mail: lisa.sorbera@thomsonreuters.com.

Although pulmonary hypertension was first described in 1891, it is an orphan disease and has not been given widespread attention within the medical community. The etiology of pulmonary hypertension remains unclear. However, a renewed interest in the mechanisms of pathogenesis has been generated in the last 10 years. Pulmonary hypertension is characterized by vascular remodeling, complex lumen-occluding vascular lesions (i.e., plexiform lesions), vasoconstriction and in situ thrombosis, and elevated pulmonary vascular resistance. Normal mean pulmonary arterial pressure is approximately 14 mmHg at rest. In a patient with primary pulmonary hypertension, mean blood pressure in the pulmonary artery is > 25 mmHg at rest and > 30 mmHg during exercise. This abnormally high pressure is associated with changes in the capillaries of the lungs, which produce increased resistance to blood flowing through the vessels. The interaction of endothelial cells with smooth muscle cells in the vessel wall is altered due to injury to vessels, which results in enhanced smooth muscle contraction. The consequence is a narrowing of the vessel and eventual proliferation of smooth muscle within the pulmonary artery walls. In addition, fibrosis occurs and vessels become stiff, as well as thickened; some vessels may become completely blocked. Blood clots form within the smaller arteries and right ventricular hypertrophy develops due to the extra demands placed on the heart. The overworked and enlarged right ventricle gradually becomes weak and loses its ability to pump enough blood to the lungs, and eventually may fail, resulting in death (1, 2, 6).

There is no cure for pulmonary hypertension, and until recently, treatment for patients diagnosed with pulmonary hypertension was limited. The ideal therapeutic strategy for pulmonary hypertension would be to reduce pulmonary vascular resistance, improve systemic circulation and increase right ventricular inotropy. To date, only the marketed phosphodiesterase PDE5 inhibitors meet these criteria. Anticoagulants are available to reduce the tendency for clots, as well as diuretics which decrease the amount of fluid in the body, thereby reducing right ventricular preload. However, diuretics are associated with the risk of systemic hypotension, renal insufficiency and syncope. Pulmonary vasodilators have become more readily available in the past 15 years, with calcium channel blockers showing particular efficacy in relaxing vascular smooth muscle and cardiac muscle. Prostacyclin, also a vasodilator, helps the blood vessels to dilate and prevents blood clots from forming. Not all patients respond equally well to the same drugs and combination therapy is being imple-

mented more and more. However, there remains a need for more effective therapies for pulmonary hypertension (1, 7-10).

The search for effective treatment strategies for pulmonary hypertension continues, with research focusing on the identification of novel targets for drug development. Those targets which are currently under active investigation are discussed below (see Figure 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

## TARGETS

### BCR/ABL

BCR/ABL is a fusion protein that possesses serine/threonine kinase activity and is encoded by both the *BCR* (breakpoint cluster region protein) and *ABL* genes. It is a result of translocation between chromosomes 22 and 9, also called the Philadelphia chromosome, and is a GTPase-activating protein for p21rac. BCR/ABL upregulates cell proliferation, decreases apoptosis and activates signaling pathways such as proto-oncogene c-Myc, HRas, Raf, signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), and thus, inhibitors may be effective in the treatment of various cancers. Recently, inhibitors of BCR/ABL kinase have also been suggested to be effective for the treatment of pulmonary hypertension. Standard treatment for this disease includes targeting endothelial dysfunction and the increased vasomotor tone. However, targeting BCR/ABL kinase may be effective in inhibiting vascular proliferation and promoting vascular apoptosis, which could be effective in attenuating the vascular remodeling typical of pulmonary hypertension (11-13).

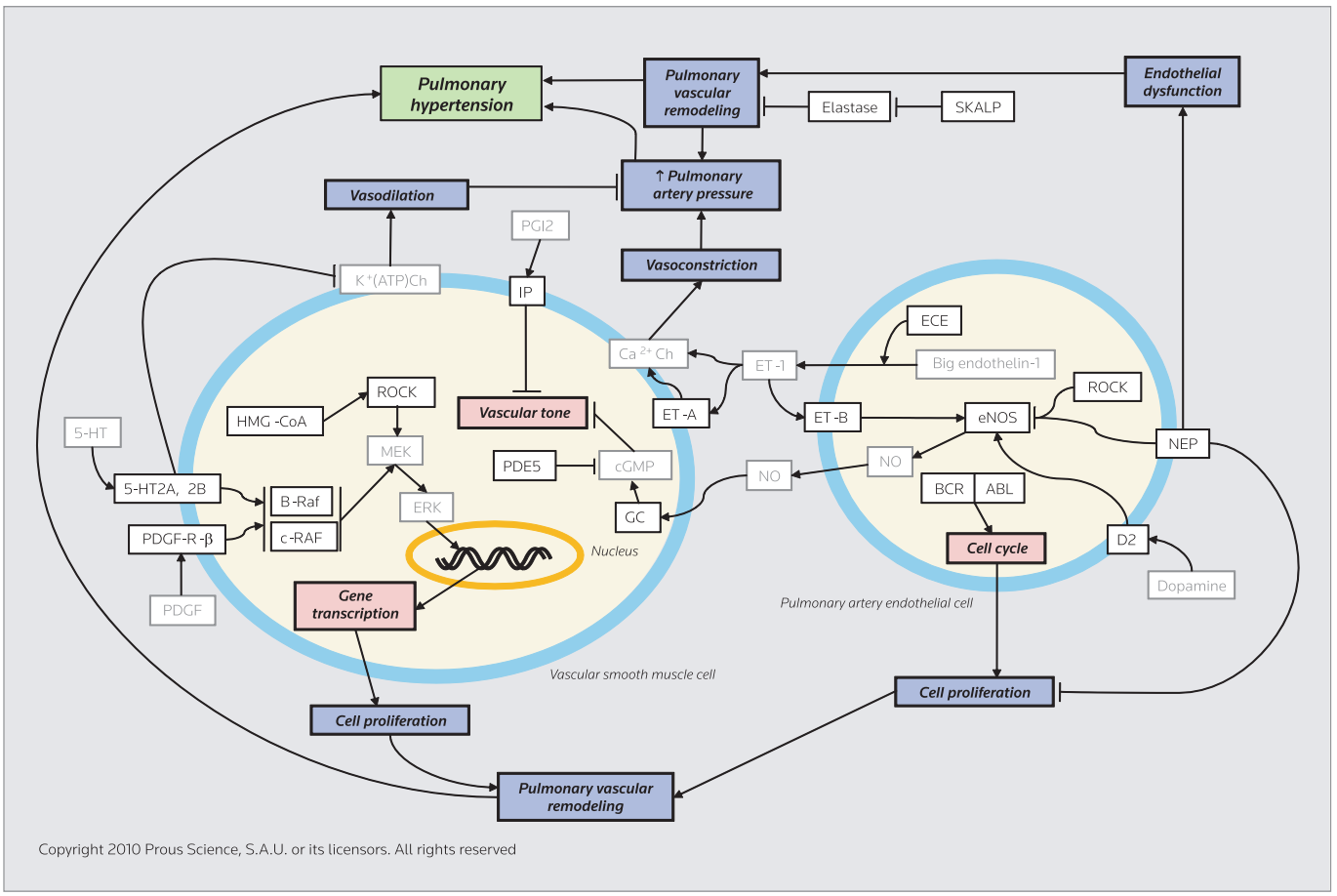




**Figure 1.** Pulmonary hypertension targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of pulmonary hypertension and their biological actions. Gray or lighter symbols are targets that are not validated (i.e., targets not associated with a product that is currently under active development for pulmonary hypertension). Abbreviations: ABL, tyrosine-protein kinase ABL; BCR, breakpoint cluster region protein; $Ca^{2+}$ Ch, calcium channel; cGMP, 3′,5′-cyclic guanosine monophosphate; D2, dopamine $D_2$ receptor; ECE, endothelin-converting enzyme; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ET-A, endothelin $ET_A$ receptor; ET-B, endothelin $ET_B$ receptor; ERK, extracellular signal-regulated kinase; GC, guanylate cyclase; HMG-CoA, HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A reductase); 5-HT, 5-hydroxytryptamine (serotonin); 5-HT2A, 2B, 5-$HT_2$ receptor; IP, prostanoid $IP_1$ receptor; $K^+$(ATP)Ch, potassium ATP channel; MEK, mitogen-activated protein kinase kinase; NEP, neprilysin; PDE5, phosphodiesterase 5 (cGMP-specific 3′,5′-cyclic phosphodiesterase); PDGF, platelet-derived growth factor; PDGF-R-β, platelet-derived growth factor receptor beta; PGI2, prostacyclin (prostaglandin $I_2$); ROCK, rho-associated protein kinase; SKALP, elafin.

***Table I.*** *Selected targets and products launched or being actively investigated for pulmonary hypertension (from Thomson Reuters Integrity℠).*

| Target name | Product | Source | Phase |
|---|---|---|---|
| BCR/ABL | Imatinib mesylate | Novartis | III |
| | Nilotinib | Novartis | II |
| Dopamine $D_2$ receptor | Terguride | Ergonex Pharma/Pfizer | II |
| Elastase | Elafin | Proteo-Biotech | I |
| Endothelial nitric oxide synthase | Beraprost sodium | Toray | L2000 |
| Endothelin $ET_A$ receptor | Bosentan | Actelion/Genentech | L2001 |
| | Sitaxentan sodium | Encysive Pharmaceuticals | L2006 |
| | Ambrisentan | Gilead | L2007 |
| | Macitentan | Actelion | III |
| | BQ-123 | Brigham & Women's Hospital | II |
| Endothelin $ET_B$ receptor | Bosentan | Actelion/Genentech | L2001 |
| | Macitentan | Actelion | III |
| | Tezosentan disodium | Actelion | II |
| Endothelin-converting enzyme | Daglutril | Solvay | II |
| Guanylate cyclase | Riociguat | Bayer | III |
| HMG-CoA reductase | Simvastatin | Imperial College | I/II |
| 5-$HT_{2A/2B}$ receptor | Terguride | Ergonex Pharma/Pfizer | II |
| Neprilysin | Daglutril | Solvay | II |
| Phosphodiesterase PDE5 | Sildenafil citrate | Pfizer | L2005 |
| | Tadalafil | Nippon Shinyaku/United Therapeutics | L2009 |
| | Udenafil | Dong-A | II |
| | PF-489791 | Pfizer | II |
| Platelet-derived growth factor beta | Sorafenib | Bayer | I |
| Prostacyclin | Epoprostenol sodium | GlaxoSmithKline | L-1998 |
| | Treprostinil sodium | United Therapeutics | L-2002 |
| | Iloprost | Bayer Schering Pharma | L-2004 |
| Prostanoid $IP_1$ receptor | Selexipag | Actelion | III |
| Proto-oncogene c-RAF | Sorafenib | Bayer | I |
| Proto-oncogene B-Raf | Sorafenib | Bayer | I |
| Rho-associated protein kinase | Fasudil hydrochloride | Asahi Kasei | II |

### Dopamine $D_2$ receptor

The dopamine $D_2$ receptor is a G protein-coupled, seven-transmembrane-spanning receptor protein ($G_i/G_o$) that binds dopamine present in the central nervous system in basal ganglia. The $D_2$ receptor inhibits cAMP synthesis by coupling to $G_{\alpha i/o}$ and also regulates $Ca^{2+}$ and potassium ion channels via phospholipase C when it forms hetero-oligomers, particularly with the $D_1$ receptor. This $D_1$–$D_2$ receptor hetero-oligomer has been proposed to facilitate a distinctive dopamine-mediated $Ca^{2+}$ signal, with important effects on synaptic plasticity. Full agonists or pure $D_2$ receptor antagonists may not be optimal therapeutic approaches due to their inability to restore the aberrant dopamine pathways to a normal level of basal tone. On the other hand, $D_2$ receptor partial agonists may stabilize activity in dopamine pathways by dampening excessive and/or restoring deficient $D_2$ receptor stimulation and achieving a desired level of basal activity. It has been speculated that a reduction in dopamine receptor function increases reactive oxygen species (ROS) activity, which may play a role in the pathogenesis of pulmonary hypertension. Modulation of the $D_2$ receptor may therefore be effective in the treatment of pulmonary hypertension (14-16).

### Elafin

Elafin (skin-derived antileukoproteinase, SKALP) is a serine and neutrophil elastase inhibitor produced in airways epithelial cells that protects these same cells from neutrophil elastase. Upregulated serine elastase activity results in increased vascular smooth muscle cell proliferation and is observed in pulmonary hypertension and chronic obstructive pulmonary disease (COPD). Elafin possesses the endogenous function of conferring anti-neutrophil elastase protection at inflammatory sites, and suppression of elastase activity could therefore increase airways inflammation and the resulting lung damage. On the other hand, activation of this enzyme could inhibit serine elastase activity and may be an effective therapy for pulmonary hypertension and COPD (11, 13, 17-19).

### Elastase

Elastases are a family of serine proteases that hydrolyze elastin. Neutrophil elastase (EC 3.4.21.37) is a major component of lung elastolytic activity and a stimulant of mucus secretion. It also induces the release of IL-8 from epithelial cells and may therefore

***Table II.*** *Selected patents for targets being validated for pulmonary hypertension (from Thomson Reuters Integrity℠).*

| Target | Patent | Source | Phase |
|---|---|---|---|
| Elastase | WO 2008003412 | Bayer Healthcare | Biological testing |
| | WO 2009080199 | Bayer Schering Pharma | Biological testing |
| | WO 2009135599 | Bayer Schering Pharma | Biological testing |
| | WO 2010078953 | Bayer Schering Pharma | Biological testing |
| Endothelin receptor | WO 1996026195 | Pfizer | Preclinical |
| | WO 1997010214 | Shionogi & Co. | Preclinical |
| | WO 2008088727 | Concert Pharmaceuticals | Biological testing |
| | WO 2008097468 | Concert Pharmaceuticals | Biological testing |
| | WO 2009141167 | Synthon | Biological testing |
| 5-$HT_2$ receptor | WO 2004089312 | Epix Pharmaceuticals | Biological testing |
| | WO 2005063220 | University of Texas System/Gilead Colorado | Biological testing |
| | WO 2005063712 | Gilead Colorado | Biological testing |
| | WO 2006034511 | Epix Pharmaceuticals | Biological testing |
| | WO 2008061968 | Boehringer Ingelheim Pharma | Biological testing |
| | WO 2009016225 | GlaxoSmithKline | Biological testing |
| | WO 2009016227 | GlaxoSmithKline | Biological testing |
| | WO 2010080357 | Boehringer Ingelheim Pharma | Biological testing |
| Phosphodiesterase PDE5A | EP 1953159 | Nycomed | Biological testing |
| | WO 1999028319 | Mochida Pharmaceutical | Biological testing |
| | WO 2003020724 | Schering (Merck & Co.) | Biological testing |
| | WO 2008004796 | SK Chemicals | Biological testing |
| | WO 2009000798 | Solvay Pharmaceuticals | Biological testing |
| | WO 2009106531 | Nycomed | Biological testing |
| | WO 2010015585 | Nycomed | Biological testing |
| | WO 2010015586 | Nycomed | Biological testing |
| | WO 2010015587 | Nycomed | Biological testing |
| | WO 2010015588 | Nycomed | Biological testing |
| | WO 2010015589 | Nycomed | Biological testing |
| | WO 2010066111 | Topharman Shanghai/Shanghai Institute Materia Medica | Biological testing |
| | WO 2010095849 | Dong-A Pharmaceutical | Biological testing |
| Prostanoid $IP_1$ receptor | WO 2008131858 | Bayer Healthcare | Biological testing |
| | WO 2008131859 | Bayer Healthcare | Biological testing |
| | WO 2009117095 | Arena Pharmaceuticals | Biological testing |
| | WO 2010068242 | Arena Pharmaceuticals. | Biological testing |
| | WO 2010077275 | Arena Pharmaceuticals | Biological testing |

prolong inflammation. Macrophage elastase, or MMP-12 (EC 3.4.24.65), hydrolyzes soluble and insoluble elastin and specifically cleaves 14-Ala-|-Leu-15 and 16-Tyr-|-Leu-17 in the insulin B chain. Inhibition of elastase is a potential target for the treatment of pulmonary hypertension, COPD, acute respiratory distress syndrome and cystic fibrosis (11, 17, 20).

### Endothelial nitric oxide synthase (eNOS)

eNOS (EC 1.14.13.39) is one of three enzymes (i.e., iNOS, nNOS) that synthesize nitric oxide (NO), L-arginine and ROS. It is constitutive and regulated by calcium/calmodulin and is involved in regulating vascular function. It is also involved in the production of ROS through modulation of Rho-associated protein kinases (ROCK), protein kinase C, voltage-gated potassium channels and ryanodine receptors, and consequently causes contraction of pulmonary artery smooth muscle cells. Increases in ROS participate in hypoxia-induced responses and may be involved in the development of pulmonary hypertension. On the other hand, deficiencies in eNOS are thought to contribute to the development of pulmonary hypertension and enhancers of eNOS expression may be an effective therapeutic option (21-26).

### Endothelin-converting enzyme (ECE)

ECE (EC 3.4.24.71) is an integral membrane protein belonging to the family of metalloproteinases. It is involved in the biosynthesis of endothelin-1 (ET-1) from its precursor, big endothelin-1. ECE-1 is the main enzyme responsible for ET-1 generation and is largely involved in blood pressure control. ECE-2 is a peptidase that converts big endothelin-1 to ET-1. The endothelin system is activated in pulmonary hypertension and is involved in both the development and progression of the disease. Thus, antagonism of the endothelin system through blockade of ECE may be an effective therapeutic strategy for pulmonary hypertension (27-29).

### Endothelin $ET_A$ and $ET_B$ receptors

ET-1 is a vasoactive 21-amino-acid peptide produced by endothelial and inflammatory cells in the pulmonary and systemic circulation

via the action of ECE. ET-1 exerts its vascular and bronchial effects through the $ET_A$ and $ET_B$ receptors. Both receptors are G protein-coupled receptors that activate the phosphatidylinositol-calcium second messenger system. However, they have distinct localization and binding affinities and activate different signaling pathways. The $ET_A$ receptor has strong affinity for ET-1, while the $ET_B$ receptor is nonspecific for ET-1, as well as ET-2 and ET-3, other vasoactive members of the endothelin family. $ET_B$ is widely distributed throughout the endothelium (e.g., lung, trachea, kidney, adrenal gland, pituitary gland, cerebellum). The endothelin system is activated in pulmonary hypertension and is involved in both the development and progression of the disease. Antagonism of the endothelin system through blockade of the $ET_A$ receptor alone or in combination with an $ET_B$ receptor antagonist may therefore be an effective therapeutic strategy (30-34).

### Guanylate cyclase

Guanylate cyclase (EC 4.6.1.2, also known as guanylyl cyclase) is a lyase that catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP) and pyrophosphate. There are soluble and membrane-bound guanylate cyclases that are expressed in almost all cell types. The membrane-bound forms bind natriuretic peptides (GC-A and GC-B) or heat-stable enterotoxins (GC-D). The soluble form is activated by endogenous NO. Activated guanylate cyclase results in higher levels of cGMP, which relaxes vascular smooth muscle cells (VSMCs) and increases vasodilation. Thus, activators of guanylate cyclase would increase the production of cGMP and enable increases in smooth muscle relaxation and vasodilation, an effective therapeutic strategy for the treatment of pulmonary hypertension (10, 21, 35).

### 5-$HT_2$ receptors

5-Hydroxytryptamine (5-HT, serotonin) is a biogenic amine neurotransmitter synthesized in neurons of the raphe nucleus in the brainstem and present in high concentrations in the hypothalamus and basal ganglia. The serotonergic system innervates almost all areas of the brain and spinal cord, and mediates many behaviors, including affective state, sleep–wakefulness, feeding behavior, sexual behavior, neuroendocrine secretion and pain, among others. Moreover, it has been implicated in the pathogenesis of pulmonary hypertension, where increased levels of the neurotransmitter have been detected in patients suffering from pulmonary hypertension, and 5-HT-induced smooth muscle hyperplasia has been observed in several forms of pulmonary hypertension. 5-HT stimulates smooth muscle cell growth and migration through 5-HT receptors, of which 5-$HT_2$ is a subfamily (5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$). 5-$HT_2$ receptors are coupled to $G_q/G_{11}$ and mediate excitatory neurotransmission. Chronic exposure to 5-HT is thought to contribute to pulmonary hypertension through activation of constrictor 5-$HT_{1B}$ receptors and proliferative 5-$HT_{2B}$ receptors. Several studies suggest that 5-HT and voltage-gated potassium ($K_v$) channels play a central role in the pathogenesis of pulmonary hypertension and that activation of 5-$HT_{2A}$ receptors inhibits the $K_v$1.5 channel, which is in part responsible for the pulmonary vasoconstriction seen in pulmonary hypertension. Thus, antagonism of the 5-$HT_2$ receptor in general and/or specific inhibition of the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor subtypes could be effective in the treatment of pulmonary hypertension (36-41).

### 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase)

HMG-CoA reductase (EC 1.1.1.34) is a key enzyme that catalyzes the rate-limiting step in the biosynthetic pathway leading from mevalonate to cholesterol and isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which plays a role in protein prenylation, a crucial step in multiple cellular processes. Protein prenylation (i.e., farnesylation and geranylgeranylation) is a post-translational modification of proteins involving the addition of isoprenoids. Geranylgeranylation allows the activation of the small GTP-binding proteins Rho and Rac. Activated Rho regulates the activity of nuclear transcription factors such as nuclear factor NF-kappa-B (NF-κB), controls the actin cytoskeleton and induces stress fiber formation. This affects intracellular transport, migration, membrane trafficking, messenger RNA stability and gene transcription. Farnesylation allows the activation of Ras protein. Activated Ras stimulates cytoplasmic signaling pathways such as the MAPK pathway that regulates gene transcription and thus growth, proliferation, differentiation and survival of cells. Inhibitors of HMG-CoA reductase have been reported to affect endothelial function, cell proliferation, inflammatory responses, immunological reactions, platelet function and lipid oxidation. Because pulmonary arterial hypertension is an inflammatory and vasoproliferative disease, inhibition of HMG-CoA reductase could provide anti-inflammatory and antiproliferative effects beneficial for the treatment of pulmonary hypertension. In addition, HMG-CoA reductase inhibitors have been shown to restore eNOS expression, which would also be effective in the treatment of pulmonary hypertension (11, 31, 42, 43).

### Neprilysin

Neprilysin (NEP; also known as neutral endopeptidase; EC 3.4.24.11) is an integral plasma membrane zinc metalloendopeptidase that is involved in the degradation of neuropeptides that play a role in cell growth and contraction; it is also involved in the degradation of other peptide hormones such as atrial natriuretic peptide and β-amyloid. Reduced levels of NEP have been associated with carcinogenesis, increased inflammation, neuroendocrine cell hyperplasia and the vascular remodeling seen in pulmonary hypertension. NEP may be protective against chronic hypoxic pulmonary hypertension and hypertension, possibly through its ability to attenuate smooth muscle cell proliferation (44-46).

### Phosphodiesterase PDE5

PDE5 (also known as cGMP-specific 3',5'-cyclic phosphodiesterase; EC 3.1.4.35) is a PDE isoenzyme that has relatively high affinity for cGMP and poor affinity for cAMP. The enzyme is found in many tissues, including VSMCs in the walls of systemic arteries and veins. cGMP relaxes VSMCs and increases vasodilation. Thus, inhibitors of PDE5 would attenuate the degradation of cGMP and enable increases in smooth muscle relaxation and vasodilation, an effective therapeutic strategy for the treatment of pulmonary hypertension (8, 31, 42, 47, 48).

### Platelet-derived growth factor receptor beta (PDGF-R-β)

PDGF-R-β is a membrane receptor tyrosine-protein kinase for the glycoprotein PDGF. The two receptor isoforms (PDGF-R-α and

PDGF-R-β) regulate cellular proliferation, differentiation and migration in normal cells and are widely expressed in several malignancies. Extracellular binding of PDGF stimulates the intrinsic tyrosine-protein kinase activity in the cytoplasmic portion of each subunit of the receptor, resulting in transphosphorylation of specific tyrosine residues. These phosphotyrosines can then serve as binding sites for intracellular signaling molecules by means of their Src homology 2 domains, thus activating multiple downstream pathways, including phosphatidylinositol-3 kinase (PI3K), phospholipase C-gamma (PLC-γ), tyrosine-protein kinase Src, Janus kinase (JAK)/STAT and MAPK pathways. PDGFs are suspected to induce pathological mesenchymal responses and PDGF-R inhibitors have been shown to inhibit cell proliferation and survival. Antagonism of this receptor type may also be effective in the treatment of pulmonary hypertension (49-52).

### Prostacyclin ($PGI_2$, prostaglandin $I_2$) and the prostanoid $IP_1$ receptor (prostacyclin receptor)

$PGI_2$ is an active product of arachidonic acid metabolism and a member of the eicosanoid family that is released by mast cells and vascular endothelium. It inhibits platelet aggregation and the vasoconstricting effects of angiotensin. It stimulates the release of renin and causes vasodilation and increases vascular permeability. The actions of $PGI_2$ are mediated via the prostanoid $IP_1$ receptor. Agonists of this receptor and prostacyclin analogues would cause vasodilation, thus reducing pulmonary arterial pressure, and therefore be effective in the treatment of pulmonary hypertension (17, 31, 53, 54).

### Proto-oncogenes B-Raf and c-RAF

B-Raf and c-RAF are serine/threonine-protein kinases that are involved in regulating cellular signal transduction pathways in response to a wide variety of external stimuli. Upstream activation of Ras leads to B-Raf and c-RAF relocalization to the membrane, where they in turn phosphorylate and activate downstream kinases in the MAPK signaling pathway. Independently of their signaling to mitogen-activated protein kinase kinase (MEK) and ERK, B-Raf and c-RAF also protect cells from apoptosis by translocating to the mitochondria, where they bind Bcl-2 and displace BAD. Because it acts as a negative regulator of apoptosis, inhibition of c-RAF could serve as a treatment option for various types of cancer. Moreover, the extensive vascular remodeling and proliferation observed in pulmonary hypertension can be compared to that observed in malignant tumors. Both involve activated growth factor and kinase signaling. Thus, inhibition of these kinases may be effective in the treatment of aberrant vascular remodeling seen in pulmonary hypertension (49, 55, 56).

### Rho-associated protein kinase

The Rho-associated protein kinases ROCK-1 and ROCK-2 are serine/threonine-specific protein kinases involved in the RhoA/Rho-associated kinase signaling pathway that regulates the state of phosphorylation of myosin phosphatase. ROCK is activated by GTP-bound RhoA and phosphorylates many substrate proteins, thereby controlling a wide variety of cellular functions, including smooth muscle contraction and proliferation, angiogenesis and synaptic remodeling. ROCK regulates assembly of the actin cytoskeleton, promoting the formation of stress fibers and focal adhesion complexes. Activation of the ROCK signaling pathway has been implicated in the vascular remodeling observed in pulmonary hypertension. In particular, ROCK signaling mediates pulmonary arterial smooth muscle cell proliferation, inflammatory cell recruitment and motility regulation. Thus, inhibition of ROCK may be an effective therapeutic strategy for the treatment of pulmonary hypertension (10, 31, 35, 57, 58).

## DISCLOSURES

The authors state no conflicts of interest.

## REFERENCES


1. Thomson Reuters Integrity Disease Briefings: Pulmonary Hypertension (online publication). Updated 2010.
2. Toshner, M., Tajsic, T., Morrell, N.W. *Pulmonary hypertension: Advances in pathogenesis and treatment*. Br Med Bull 2010, 94: 21-32.
3. Stringham, R., Shah, N.R. *Pulmonary arterial hypertension: An update on diagnosis and treatment*. Am Fam Physician 2010, 82(4): 370-7.
4. Gladwin, M.T., Ghofrani, H.A. *Update on pulmonary hypertension 2009*. Am J Respir Crit Care Med 2010, 181(10): 1020-6.
5. Escribano Subias, P., Barberà Mir, J.A., Suberviola, V. *Current diagnostic and prognostic assessment of pulmonary hypertension*. Rev Esp Cardiol 2010, 63(5): 583-96.
6. Rosenblum, W.D. *Pulmonary arterial hypertension: Pathobiology, diagnosis, treatment, and emerging therapies*. Cardiol Rev 2010, 18(2): 58-63.
7. Adamali, H., Gaine, S.P., Rubin, L.J. *Medical treatment of pulmonary arterial hypertension*. Semin Respir Crit Care Med 2009, 30(4): 484-92.
8. Archer, S.L., Michelakis, E.D. *Phosphodiesterase type 5 inhibitors for pulmonary arterial hypertension*. N Engl J Med 2009, 361(19): 1864-71.
9. Macchia, A., Marchioli, R., Tognoni, G., Scarano, M., Marfisi, R., Tavazzi, L., Rich, S. *Systematic review of trials using vasodilators in pulmonary arterial hypertension: Why a new approach is needed*. Am Heart J 2010, 159(2): 245-57.
10. Murthy, S.N., Nossaman, B.D., Kadowitz, P.J. *New approaches to the treatment of pulmonary hypertension: From bench to bedside.* Cardiol Rev 2010, 18(2): 76-84.
11. Stenmark, K.R., Rabinovitch, M. *Emerging therapies for the treatment of pulmonary hypertension*. Pediatr Crit Care Med 2010, 11(2, Suppl.): S85-90.
12. Ali, O., Wharton, J., Gibbs, J.S., Howard, L., Wilkins, M.R. *Emerging therapies for pulmonary arterial hypertension*. Expert Opin Investig Drugs 2007, 16(6): 803-18.
13. Jeffery, T.K., Wanstall, J.C. *Pulmonary vascular remodeling: A target for therapeutic intervention in pulmonary hypertension.* Pharmacol Ther 2001, 92(1): 1-20.
14. Staropoli, J.F., Xin, W., Sims, K.B. *Co-segregation of Norrie disease and idiopathic pulmonary hypertension in a family with a microdeletion of the NDP region at Xp11.3-p11.4.* J Med Genet 2010, 47(11): 786-90.
15. Zeng, C., Villar, V.A., Yu, P., Zhou, L., Jose, P.A. *Reactive oxygen species and dopamine receptor function in essential hypertension*. Clin Exp Hypertens 2009, 31(2): 156-78.
16. Zeng, C., Armando, I., Luo, Y., Eisner, G.M., Felder, R.A., Jose, P.A. *Dysregulation of dopamine-dependent mechanisms as a determinant of hypertension: Studies in dopamine receptor knockout mice*. Am J Physiol Heart Circ Physiol. 2008, 294(2): H551-69.

17. Rose, F., Hattar, K., Gakisch, S. et al. *Increased neutrophil mediator release in patients with pulmonary hypertension—Suppression by inhaled iloprost*. Thromb Haemost 2003, 90(6): 1141-9.
18. Zaidi, S.H., You, X.M., Ciura, S., Husain, M., Rabinovitch, M. *Overexpression of the serine elastase inhibitor elafin protects transgenic mice from hypoxic pulmonary hypertension*. Circulation 2002, 105(4): 516-21.
19. Cowan, K.N., Heilbut, A., Humpl, T., Lam, C., Ito, S., Rabinovitch, M. *Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor*. Nat Med 2000, 6(6): 698-702.
20. Loukanov, T., Geiger, R., Agrawal, R. *Animal models related to congenital heart disease and clinical research in pulmonary hypertension*. Cardiology 2010, 116(1): 18-25.
21. Wolin, M.S., Gupte, S.A., Mingone, C.J., Neo, B.H., Gao, Q., Ahmad, M. *Redox regulation of responses to hypoxia and NO-cGMP signaling in pulmonary vascular pathophysiology.* Ann NY Acad Sci 2010, 1203: 126-32.
22. Umar, S., Steendijk, P., Ypey, D.L., Atsma, D.E., van der Wall, E.E., Schalij, M.J., van der Laarse, A. *Novel approaches to treat experimental pulmonary arterial hypertension: A review.* J Biomed Biotechnol 2010, 2010: 702836.
23. Perez-Vizcaino, F., Cogolludo, A., Moreno, L. *Reactive oxygen species signaling in pulmonary vascular smooth muscle.* Respir Physiol Neurobiol 2010, Epub ahead of print.
24. Fike, C.D., Pfister, S.L., Slaughter, J.C. et al. *Protein complex formation with heat shock protein 90 in chronic hypoxia-induced pulmonary hypertension in newborn piglets.* Am J Physiol Heart Circ Physiol 2010, 299(4): H1190-204.
25. Xu, X.F., Ma, X.L., Shen, Z., Wu, X.L., Cheng, F., Du, L.Z. *Epigenetic regulation of the endothelial nitric oxide synthase gene in persistent pulmonary hypertension of the newborn rat*. J Hypertens 2010, 28(11): 2227-35.
26. Zhao, Y.Y., Malik, A.B. *A novel insight into the mechanism of pulmonary hypertension involving caveolin-1 deficiency and endothelial nitric oxide synthase activation.* Trends Cardiovasc Med 2009, 19(7): 238-42.
27. Dai, Z.K., Hsieh, C.C., Chai, C.Y. et al. *Protective effects of a dual endothelin converting enzyme/neutral endopeptidase inhibitor on the development of pulmonary hypertension secondary to cardiac dysfunction in the rat.* Pediatr Pulmonol 2010, 45(11): 1076-85.
28. Schulz, H., Dale, G.E., Karimi-Nejad, Y., Oefner, C. *Structure of human endothelin-converting enzyme I complexed with phosphoramidon.* J Mol Biol 2009, 385(1): 178-87.
29. Navarrete, C.T., Devia, C., Lessa, A.C. et al. *The role of endothelin converting enzyme inhibition during group B Streptococcus-induced pulmonary hypertension in newborn piglets.* Pediatr Res 2003, 54(3): 387-92.
30. Mercier, O., Sage, E., Izziki, M., Humbert, M., Dartevelle, P., Eddahibi, S., Fadel, E. *Endothelin A receptor blockade improves regression of flow-induced pulmonary vasculopathy in piglets.* J Thorac Cardiovasc Surg 2010, 140(3): 677-83.
31. Blaukovitsch, M., Zabel, P., Hauber, H.P. *Vasoproliferation and antiproliferative treatment options in pulmonary arterial hypertension.* Recent Pat Cardiovasc Drug Discov 2009, 4(2): 142-9.
32. Sauvageau, S., Thorin, E., Villeneuve, L., Dupuis, J. *Change in pharmacological effect of endothelin receptor antagonists in rats with pulmonary hypertension: Role of ETB-receptor expression levels*. Pulm Pharmacol Ther 2009, 22(4): 311-7.
33. Pullamsetti, S.S., Schermuly, R.T. *Endothelin receptor antagonists in preclinical models of pulmonary hypertension*. Eur J Clin Invest 2009, 39(Suppl. 2): 3-13.
34. Janosi, T., Peták, F., Fontao, F., Morel, D.R., Beghetti, M., Habre, W. *Differential roles of endothelin-1 ETA and ETB receptors and vasoactive intestinal polypeptide in regulation of the airways and the pulmonary vasculature in isolated rat lung.* Exp Physiol 2008, 93(11): 1210-9.
35. Casey, D.B., Badejo, A.M., Dhaliwal, J.S. et al. *Analysis of responses to the Rho-kinase inhibitor Y-27632 in the pulmonary and systemic vascular bed of the rat.* Am J Physiol Heart Circ Physiol 2010, 299(1): H184-92.
36. Esteve, J.M., Launay, J.M., Kellermann, O., Maroteaux, L. *Functions of serotonin in hypoxic pulmonary vascular remodeling*. Cell Biochem Biophys 2007, 147(1): 33-44.
37. Eddahibi, S., Adnot, S. *The serotonin pathway in pulmonary hypertension*. Arch Mal Coeur Vaiss 2006, 99(6): 621-5.
38. Kaumann, A.J., Levy, F.O. *5-Hydroxytryptamine receptors in the human cardiovascular system*. Pharmacol Ther 2006, 111(3): 674-706.
39. Cogolludo, A., Moreno, L., Lodi, F., Frazziano, G., Cobeño, L., Tamargo, J., Perez-Vizcaino, F. *Serotonin inhibits voltage-gated K+ currents in pulmonary artery smooth muscle cells: Role of 5-HT2A receptors, caveolin-1, and KV1.5 channel internalization.* Circ Res 2006, 98(7): 931-8.
40. Marcos, E., Fadel, E., Sanchez, O. et al. *Serotonin-induced smooth muscle hyperplasia in various forms of human pulmonary hypertensio*n. Circ Res 2004, 94(9): 1263-70.
41. Hervé, P., Launay, J.M., Scrobohaci, M.L. et al. *Increased plasma serotonin in primary pulmonary hypertension*. Am J Med 1995, 99(3): 249-54.
42. Satoh, M., Satoh, A. *3-Hydroxy-3-methylglutaryl (HMG)-COA reductase inhibitors and phosphodiesterase type V inhibitors attenuate right ventricular pressure and remodeling in a rat model of pulmonary hypertension*. J Pharm Pharm Sci 2009, 11(2): 118s-30s.
43. Guerard, P., Rakotoniaina, Z., Goirand, F., Rochette, L., Dumas, M., Lirussi, F., Bardou, M. *The HMG-CoA reductase inhibitor, pravastatin, prevents the development of monocrotaline-induced pulmonary hypertension in the rat through reduction of endothelial cell apoptosis and overexpression of eNOS.* Naunyn Schmiedebergs Arch Pharmacol 2006, 373(6): 401-14.
44. Wick, M.J., Buesing, E.J, Wehling, C.A. et al. *Decreased neprilysin and pulmonary vascular remodeling in chronic obstructive pulmonary disease*. Am J Respir Crit Care Med 2010, Epub ahead of print.
45. Dempsey, E.C., Wick, M.J., Karoor, V. et al. *Neprilysin null mice develop exaggerated pulmonary vascular remodeling in response to chronic hypoxia*. Am J Pathol 2009, 174(3): 782-96.
46. Fitzpatrick, P.A., Guinan, A.F., Walsh, T.G. et al. *Down-regulation of neprilysin (EC3.4.24.11) expression in vascular endothelial cells by laminar shear stress involves NADPH oxidase-dependent ROS production*. Int J Biochem Cell Biol 2009, 41(11): 2287-94.
47. Francis, S.H., Busch, J.L., Corbin, J.D., Sibley, D. *cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action.* Pharmacol Rev 2010, 62(3): 525-63.
48. Jin, B., Luo, X.P., Ni, H.C., Shi, H.M. *Phosphodiesterase type 5 inhibitors for high-altitude pulmonary hypertension: A meta-analysis.* Clin Drug Investig 2010, 30(4): 259-65.
49. Agbani, E.O., Coats, P., Mills, A., Wadsworth, R.M. *Peroxynitrite stimulates pulmonary artery endothelial and smooth muscle cell proliferation: Involvement of ERK and PKC*. Pulm Pharmacol Ther 2010, Epub ahead of print.
50. Cohen, E.D., Ihida-Stansbury, K., Lu, M.M., Panettieri, R.A., Jones, P.L., Morrisey, E.E. *Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway.* J Clin Invest 2009, 119(9): 2538-49.
51. Andrae, J., Gallini, R., Betsholtz, C. *Role of platelet-derived growth factors in physiology and medicine*. Genes Dev 2008, 22(10): 1276-312.
52. Liu, Y., Li, M., Warburton, R.R., Hill, N.S., Fanburg, B.L. *The 5-HT transporter transactivates the PDGFbeta receptor in pulmonary artery smooth muscle cells.* FASEB J 2007, 21(11): 2725-34.

53. Kumar, V.H., Swartz, D.D., Rashid, N., Lakshminrusimha, S., Ma, C., Ryan, R.M., Morin, F.C. 3rd. *Prostacyclin and milrinone by aerosolization improve pulmonary hemodynamics in newborn lambs with experimental pulmonary hypertension.* J Appl Physiol 2010, 109(3): 677-84.

54. Martin, K.A., Gleim, S., Elderon, L., Fetalvero, K., Hwa, J. *Chapter 6 the human prostacyclin receptor from structure function to disease*. Prog Mol Biol Transl Sci 2009, 89: 133-66.

55. Moreno-Vinasco, L., Garcia, J.G. *Receptor tyrosine kinase inhibitors in rodent pulmonary hypertension.* Adv Exp Med Biol 2010, 661: 419-34.

56. Klein, M., Schermuly, R.T., Ellinghaus, P. et al. *Combined tyrosine and serine/threonine kinase inhibition by sorafenib prevents progression of experimental pulmonary hypertension and myocardial remodeling.* Circulation 2008, 118(20): 2081-90.

57. Connolly, M.J., Aaronson, P.I. *Key role of the RhoA/Rho kinase system in pulmonary hypertension.* Pulm Pharmacol Ther 2010, Epub ahead of print.

58. Li, X.H., Peng, J., Tan, N., Wu, W.H., Li, T.T., Shi, R.Z., Li, Y.J. *Involvement of asymmetric dimethylarginine and Rho kinase in the vascular remodeling in monocrotaline-induced pulmonary hypertension.* Vascul Pharmacol 2010, 53(5-6): 223-9.