REVIEW

Novel Experimental Therapies for Treatment of Pulmonary Arterial Hypertension

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Abstract: Pulmonary arterial hypertension (PAH) is a progressive and devastating disease characterized by pulmonary artery vasoconstriction and vascular remodeling leading to vascular rarefaction with elevation of pulmonary arterial pressures and pulmonary vascular resistance. Often PAH will cause death from right heart failure. Current PAH-targeted therapies improve functional capacity, pulmonary hemodynamics and reduce hospitalization. Nevertheless, today PAH still remains incurable and is often refractory to medical therapy, underscoring the need for further research. Over the last three decades, PAH has evolved from a disease of unknown pathogenesis devoid of effective therapy to a condition whose cellular, genetic and molecular underpinnings are unfolding. This article provides an update on current knowledge and summarizes the progression in recent advances in pharmacological therapy in PAH.

Keywords: pulmonary hypertension, investigational drugs, vascular remodelling, animal models, clinical trials, new drug targets, toxicity

Introduction

Pulmonary hypertension (PH) is a vascular disease characterized by progressive increase in pulmonary pressures, ultimately leading to death from right heart failure.[1]

In 2018, the sixth World Symposium on Pulmonary Hypertension has recommended considering a new hemodynamic definition of PH to be mean pulmonary artery pressure at rest of greater than 20 mmHg.[2]

PH may occur as a consequence of pulmonary vascular disease, chronic left heart or lung disease, pulmonary embolism, or other etiologies.[3] The World Symposium on PH has led to an internationally accepted classification of PH in five groups: Group 1 comprises pulmonary arterial hypertension (PAH), Group 2 comprises PH associated with left heart disease, Group 3 consists of PH associated with lung disease and/or hypoxia, Group 4 includes chronic thromboembolic PH, and ultimately, Group 5 constitutes of PH due to various causes including hemolytic anemia, sarcoidosis, histiocytosis X, glycogen storage disease, and renal diseases.[3]

Group 1 PAH can also be classified into many subcategories including idiopathic PAH (IPAH), heritable PAH (HPAH), and PAH associated with other diseases, such as connective tissue diseases, liver cirrhosis, congenital heart malformations, HIV infection, and schistosomiasis.[3] All types of PAH share common pathological changes, such as pulmonary artery endothelial cell (PAEC) dysfunction and proliferation; pulmonary artery smooth muscle cell (PASMC) proliferation, migration, and contraction; inflammation; and fibroblast proliferation, activation, and migration.

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Vascular lesions found in the lungs of PAH patients are responsible for the increase in pulmonary vascular resistance (PVR). The proliferative and fibrotic PAH lesions mainly concern small lung vessels. Precapillary lesions are typically located in muscular arteries with diameters less than 500 um diameter and arterioles.

The main histopathological characteristic of PAH is vascular wall remodeling, which comprises intima proliferation, medial and adventitial layer hypertrophy, as well as extracellular matrix deposition.[4]

In severe cases of PAH, plexiform lesions are found. Plexiform lesions, glomeruloid-like structures, are typically located at branching points of muscular arteries and consist of a network of vascular channels lined by endothelial cells and a core of myofibroblastic or less differentiated cells.[5]

The pathobiology of PAH is complex, involving crosstalk between several signaling pathways that comprises metabolic shift (Warburg effect), humoral modulation, impaired angiogenesis with loss of distal vessels, growth factors (eg, PDGF), and chronic inflammation.[6–17]

PAEC dysfunction is considered to be a critical initiating factor in the pathobiology of PAH.[18] PAEC dysfunction observed in PAH includes an imbalance in the secretion of vasodilators and anti-mitogenic factors such as nitric oxide and prostacyclin and vasoconstrictors and pro-mitogenic molecules such as endothelin.[19]

Endothelial dysfunction also leads to the production of factors affecting PASMCs, thrombotic mediators and inflammatory cytokines.[19] As a result, there is migration and proliferation of PASMCs into the small precapillary pulmonary arterioles, which normally lack a smooth muscle layer. Abnormal presence of myofibroblasts is also seen.[20] The proliferation of PASMCs and myofibroblasts will lead to luminal narrowing and diminished ability for the vessel to dilate appropriately, ultimately causing elevation of the pulmonary arterial pressures and PVR.

Additionally, PAECs display alteration in energy production with a shift to anaerobic glycolysis, lactate synthesis and reduced oxidative phosphorylation.[21] In PASMCs, there is evidence of proliferation and apoptosis resistance due to genetically[22] controlled mechanisms, metabolic reprogramming,[23] calcium mishandling,[24] and abnormal mitochondrial function.[25] Mitochondrial dysfunction creates a Warburg effect that will stimulate proliferation of vascular fibroblasts.[26] Extracellular matrix (ECM) remodeling promotes PAH by reducing pulmonary arterial

compliance. Finally, the expanding body of evidence has associated PAH with a substantial inflammatory process with infiltration of T- and B-lymphocytes as well as elevation of circulating inflammatory cytokines[27] [Figure 1].

Available current FDA-approved pharmacotherapies to treat PAH fall into three main therapeutic groups: prostacyclins [cyclic adenosine monophosphate (cAMP) pathway], phosphodiesterase (PDE) 5-inhibitors and soluble guanylate cyclase stimulators [nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway], and endothelin receptor antagonists [endothelin-1 (ET-1) pathway]. Used as single agent or in combination, these different agents have significantly improved functional status, quality of life, hemodynamics and PAH hospitalizations.[28] In the case of intravenous epoprostenol survival, these responses are usually partial and often temporary.[29] Also, these therapeutics do not target the fundamental key factors involved in the pathogenesis of the disease as they act essentially as vasodilator agents.

There is an imperative necessity, therefore, for the development of novel therapies that target key molecular pathways and will potentially cure this devastating disease.

This article presents current knowledge and summarizes the progression and recent advances in pharmacological therapy in PAH (Table 1).

Bone Morphogenetic Protein Receptor-2 (BMPR2) Signaling

Aberrant bone morphogenetic protein (BMP) and, more specifically dysfunctional bone morphogenetic protein receptor-2 (BMPR2) is considered to be one of the cornerstones in the PAH pathogenesis.

The BMPR2 is a membrane receptor that belongs to the TGF-β superfamily. This receptor was initially described as being involved in the regulation of cell growth and differentiation of cartilages and bones.[30,31] More recently, it was demonstrated that the BMPR2 receptor is involved in the regulation of growth and apoptosis of other cells such as PAECS and PASMCs.

BMPR2 mutations are responsible for a decrease in the receptor expression and function, leading to an abnormal proliferation of PASMCs.[32,33] This decrease may promote PAECs apoptosis resistance, development of vascular remodeling and pulmonary hypertension.[34] Thus, BMPR2 signaling appears to have a protective function by promoting the survival of PAECs, inhibiting PASMCs proliferation and triggering anti-inflammatory response.[35–37]
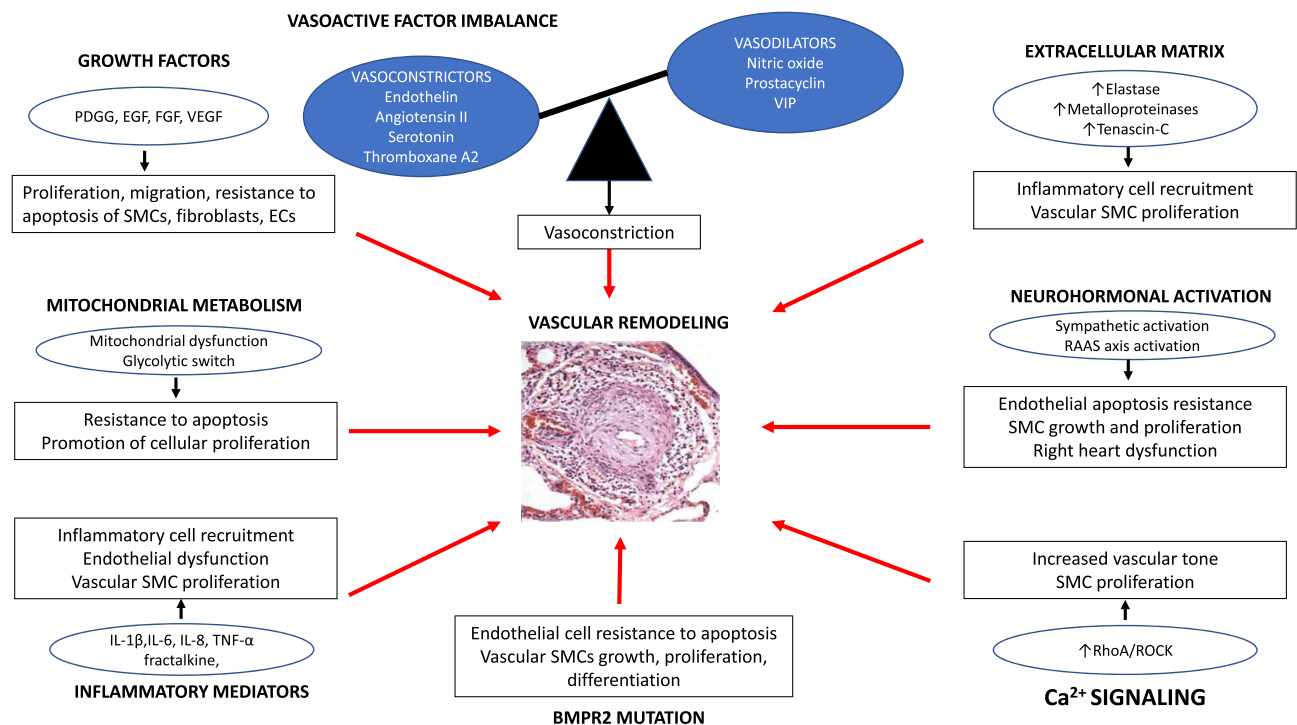
Figure 1 Physiopathological mechanisms of pulmonary arterial hypertension.
Abbreviations: ↑, increase in; ECs, endothelial cells; EGF, epidermal growth factor; FGF, fibroblast growth factor IL, interleukin; PDGF, platelet-derived growth factor RAAS, renin–angiotensi–-aldosterone system; SMCs, smooth muscle cells; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

BMPR2 ligands, the bone morphogenetic proteins (BMPs), such as BMP9 and BMP10, are cytokines regulating growth, differentiation, and apoptosis of PAECs and PASMCs. BMPs play a role in cell differentiation during embryogenesis and in the maintenance and repair of adult tissues.[38–40] Initially BMPs, such as BMP9 and BMP 10, bind and bring BMPR2 and ALK1 together, resulting in phosphorylation of cytoplasmic protein called Smads (Smad1, Smad5 Smad8). These Smad proteins then bind with Smad4 in the cytosol. This new complex is translocated into the nucleus and modulates the expression of target genes[43,44] (Figure 2).

The importance of the BMPR2 signaling pathway in the PAH pathobiology is confirmed by the observation that >80% of subjects with heritable PAH and approximately 20% of the subjects with idiopathic PAH have a gene mutation leading to BMPR2 dysfunction.[41,42] Also, subjects with no BMPR2 mutation and IPAH or PAH associated with other conditions such as connective tissue disease or HIV have reduced pulmonary vascular expression of BMPR2.[43] Estrogen also can diminish BMPR2 expression, potentially explaining the female predisposition of developing the disease compared to males.[44]

Studies from PH animal models have confirmed the involvement of BMPR2 mutation in the development of PH. West et al[45] demonstrated the development of pulmonary vascular remodeling in mice expressing a dominant negative form of BMPR2. Specifically in their PASMCs. These mice developed increased pulmonary artery pressures, pulmonary arterial muscularization and right ventricular hypertrophy (RVH). Also, Hong et al[46] showed that deletion of the *BMPR2* gene in PAECs predisposed mice to develop PH.

Even though the exact mechanism of how BMPR2 dysfunction leads to vascular remodeling remains unexplained, the BMPR2 signaling seems to play a critical role in thwarting PAECs and PASMCs proliferation from several pro-mitogenic factors such as VEGF, bFDF, hEGF, platelet-derived growth factor (PDGF),[37,47] and prevents PAECs from apoptosis.[34]

Based on all these observations and studies, regulation of the BMPR2 signaling is thought to be an important potential target for the treatment of PAH.

Therapies Targeting BMPR2 Signaling Pathway
Tacrolimus (FK506)
Tacrolimus (FK506) is an immunosuppressive drug used in allogeneic organ transplant.[48] It was shown to be a potent

Table I Summary of Clinical Trials Data of New Potential Drugs in the Treatment of PAH

	Clinical Trials.gov	Study Design	Study Duration	Inclusion Criteria	Primary Outcome	Secondary Outcomes	Status	Results	REF
Therapies targeting BMPR2 signaling									
Tacrolimus	NCT01647945	Phase II, single-center, double-blind, randomized	16-weeks	PAH	Safety	Clinical events 6MWDT NT-proBNP Echo changes	Terminated	No significant change in 6MWDT and echo parameters	[51]
Therapies targeting Inflammation and Immunity									
Tocilizumab	NCT02676947 (TRANSFORM-UK)	Phase II, open-label	6 months	PAH	Safety changes in PVR	6MWDT NT-proBNP WHO FC QOL Numbers of AEs	Completed	Results not reported	
Anakinra	NCT03057028	Pilot study, open-label, single-group	14 days	PAH	Peak VO$_2$	CRP, IL-6 NT-proBNP	Completed	No change in Peak VO$_2$ Improvement of QOL	[91]
Bardoxolone	NCT02036970 (LARIAT)	Phase II, double-blind, randomized	16 weeks	WHO PH Groups I, III or V PH	Changes in 6MWDT	–	Completed	Improvement in 6MWDT	[106]
	NCT02657356 (CATALYST)	Phase III, double-blind, randomized	24 weeks	CTD-PAH	Changes in PVR	–	Completed	Results not reported	
	NCT03068130 (RANGER) extended study in patients previously enrolled in Bardoxolone trial	Phase III, single group assignment, open-label extension	Long-term	PAH	Long-term safety	–	Active	NA	
DMF (dimethyl fumarate)	NCT02981082	Phase I, double-blind, randomized, placebo controlled	24 weeks	SSc-PAH	6MWDT	Time to clinical worsening, BORG Dyspnea Index (BDI), oxidative stress markers, BNP	Terminated	Results not reported	
Rituximab	NCT01086540	Phase II, double-blind, placebo controlled, multicenter	24 weeks	SSc-PAH	6MWDT	PVR, 6MWDT, time to clinical worsening, DLCO change, number of AEs, mortality	Completed	No significant change in 6MWDT	[110]

Therapies targeting tyrosine kinases (TK) signaling pathway

Drug	NCT	Design	Duration	Disease	Primary outcome	Secondary outcome	Status	Results	Ref
Imatinib	NCT00477269	Phase II, double-blind, placebo-controlled, multicenter	24 weeks	PAH	Safety 6MWDT	Changes in WHO class, invasive hemodynamics	Completed	No significant change in 6MWDT PVR improved	[118,120]
	NCT00902174 (IMPRES)	Phase III, Phase II, double-blind, placebo-controlled	24 weeks	PAH	6MWDT	Number of clinical worsening events, Invasive hemodynamics	Completed	Significant change in 6MWDT PVR improved	[121]
	NCT01117987 (IMPRES extension)	Single group assignment, extension	Long term	PAH	Safety (# AEs)	6MWDT Incidence of Clinical worsening	Completed	6MWDT improvement was maintained	[121]
Nilotinib	NCT01179737	Phase II, double blind, placebo controlled	24 weeks	PAH	PVR	6MWDT # AEs	Terminated	Terminated due to serious AEs	
Sorafenib	NCT00452218	Phase Ib, open-label exploratory, single-center	16 weeks	PAH	6MWD	Efficacy, WHO FC, RHC, Treadmill	Completed	Results not reported	

Therapies targeting mitochondrial dysfunction

Drug	NCT	Design	Duration	Disease	Primary outcome	Secondary outcome	Status	Results	Ref
DCA (dichloroacetate)	NCT01083524	Phase I, open-label, two-center	16 weeks	PAH	Safety	PVR, 6MWDT, RV size/frction by MRI, NT-proBNP Lung/RV metabolism	Completed	Improvement PA pressures, PVR and lung metabolism	[162]
Ranolazine	NCT01757808	Phase I, double-blind, placebo controlled	12 weeks	PAH	PVR	Change in CPET, in RV parameters, 6MWDT, AEs	Completed	Safe No HD improvement	[172]
	NCT01174173	Phase III	12 weeks	PAH	6MWDT, QOL, angina sxs	RHC parameters, RV function (echo)	Completed		[173]
	NCT01839110	Phase IV, double-blind, placebo controlled	26 weeks	PAH	Change in RVEF	–	Completed	Results not reported	[174]
Selonsertib	NCT02234141 (ARROW)	Phase II, dose-ranging, double-blind, placebo controlled	24 weeks	PAH	PVR	Invasive HD, 6MWDT, BDI, NT-proBNP, QOL, TTCW, RV echo parameters	Completed	No PVR improvement	
Trimetazidine	NCT02102672	Phase II, randomized, crossover	12 weeks	PAH	RV parameters (echo)	6MWDT, BDI, BNP, galactin-3, TTCW	Unknown		
	NCT03273387	Phase II, double-blind, placebo-controlled	12 weeks	PAH	RVEF (MRI)	Functional capacity by SF-36	Completed	Results not reported	

(*Continued*)

Table 1 (Continued).

Clinical Trials.gov	Study Design	Study Duration	Inclusion Criteria	Primary Outcome	Secondary Outcomes	Status	Results	REF
Therapies targeting oxidative stress signaling pathway								
Inhaled Nitrite — NCT01431313	Phase II, open-label, parallel-dose study	16 weeks	PAH	PVR	TTCW	Terminated	Results not reported	
Coenzyme Q10 — NCT01148836	Non-randomized, open-label, single-center	12 weeks	PAH	Echo parameters	RBCs, Hb, Htc, MCH	Completed	No change in echo parameters, 6MWDT or biomarkers	
Therapies targeting extracellular matrix								
Elafin — NCT03522935	Phase I, dose escalation, double-blind	5 weeks	Normal healthy subjects	Safety (# Aes)	PK/PD	Active, recruiting	–	
Therapies targeting metabolism								
Metformin — NCT03617458	Phase II, 2×2 factorial randomized, blinded	12 weeks	PAH	6MWDT Change in WHO FC	Weight, BMI, BDI, QOL, estradiol, BNP, RV echo parameters, mortality, hospitalization	Active, recruiting	–	
Therapies targeting neurohormonal modulation pathway								
Carvedilol — NCT01586156 (PAHTCH)	Parallel assignment, blinded, placebo controlled	24 weeks	PAH	Cardiac glucose uptake	RVSP (echo) 6MWDT NT-proBNP	Completed	No change of RV function, no change in 6MWDT	[277]
Bisoprolol — NCT01246037	Crossover, placebo-controlled, blinded, single	52 weeks	PAH	RVEF (MRI) Safety	Exercise capacity Pressure volume loop	Terminated	No benefit RVEF unchanged Decreased in 6MWDT and CI	[278]
rhACE2 (GSK2586881) — NCT03177603	Phase II, open-label, dose escalation	4 weeks	PAH	PVR CO Mean PA	# AEs, labs, Ang II, NT-proBNP, nitrite	Completed	No reduction in PVR	
Spironolactone — NCT02253394	Phase II, crossover	12 weeks	PAH	Peak VO$_2$	-	Terminated	Only 2 participants enrolled	
NCT01712620	Parallel assignment, blinded, placebo-controlled	24 weeks	PAH	6MWDT	Peak VO$_2$, RV function, biomarkers	Recruiting	–	

	NCT number	Phase/Design	Duration	Condition	Primary outcome	Secondary outcomes	Status	Results	Ref
Therapies targeting endogenous vasoactive peptides pathway									
VIP	NCT03556020	Phase II, double-blind, placebo-controlled	24 weeks	PAH	# AEs, PVR, Vital signs, EKG	6MWDT, NT-proBNP, invasive HD, BDI, WHO FC, incidence of CW, PK/PD	Recruiting	–	
Therapies targeting the serotonin system signaling pathway									
Escitalopram	NCT00190333	Phase III, double-blind, placebo-controlled	16 weeks	PAH	6MWDT	Invasive HD, NYHA, Dyspnea Scale, QOL, safety	Completed	Results not reported	
Fluoxetine	NCT00942708	Phase II, single group assignment, open-label	12 weeks	PAH	PVR	6WDT, Depression Scale	Completed	Results not reported	
Therapies targeting estrogen signaling pathway									
Anastrozole	NCT01545336	Phase II, double-blind, placebo-controlled	12 weeks	PAH	Estradiol levels, TAPSE	6MWDT	Completed	↓40% Estradiol 6MWDT improvement	[369]
	NCT03229499 (PHANTOM)	Phase II, double-blind, placebo-controlled	52 weeks	PAH	6MWDT	RV function, NT-proBNP, biomarkers, QOL, TTCW, AEs	Recruiting	–	
Tamoxifen	NCT03528902	Phase II, single-center, double-blind, placebo-controlled	24 weeks	PAH	TAPSE	6MWDT, QOL, BNP, Hb A1c	Recruiting	–	
DHEA	NCT03648385 (EDIPHY)	Phase II, double-blind, placebo-controlled	18 weeks	PAH	RV function (echo)	RVEF, NT-proBNP, sex hormone levels, 6MWDT, QOL, # AEs	Recruiting	–	
Fulvestrant	NCT02911844	Phase II, single group, open-label, single-center	9 weeks	PAH	Estradiol levels, TAPSE, 6MWDT, NT-proBNP	–	Completed	6MWDT improvement No change in TAPSE	
Therapies targeting iron deficiency									
Ferric carboxymaltose	NCT01447628	Phase II, crossover, double-blind	12 weeks	PAH	PVR, Exercise capacity	Peak VO_2, cardiac MRI, 6MWDT, WHO FC, NT proBNP, QOL, Safety	Completed	Results not reported	
Oral FeSO4	NCT01446848	Single-group, open-label	12 weeks	IPAH	Serum ferritin	6MWDT, death, hospitalization, erythropoietin, TF sat, RVSP, AEs	Completed	Results not reported	

(Continued)

Table 1 (Continued).

	Clinical Trials.gov	Study Design	Study Duration	Inclusion Criteria	Primary Outcome	Secondary Outcomes	Status	Results	REF
Therapies targeting DNA damage									
Olaparib	NCT03782818 (OPTION)	Phase I, single-group, open-label	24 weeks	PAH	# AEs	6MWDT, WHO FC, NT-proBNP, QOL	Recruiting	–	

Abbreviations: 6MWDT, 6-minute walk distance test; AEs, adverse events; Ang II, angiotensin II; BDI, BORG Dyspnea Index; BNP, brain natriuretic peptide; CI, cardiac index; CW, clinical worsening; FC, functional class; N/A, not available; HD, hemodynamics; PK/PD, pharmacokinetics/pharmacodynamics; PVR, pulmonary vascular resistance; REF, reference; RVEF, right ventricular ejection fraction; RVSP, right ventricular systolic pressure; SSc-PAH, systemic sclerosis-PAH; TF, transferrin; QOL, quality of life; TTCW, time to clinical worsening; VO₂, oxygen consumption; WHO, World Health Organization.

BMPR2 activator that reversed endothelial dysfunction in PAH patient cells and experimental PH.[49] Tacrolimus reverses pulmonary vascular remodeling, lessens right ventricular hypertrophy (RVH) and reduces right ventricular systolic pressure (RVSP) in a mouse model of chronic hypoxia.[49]

Compassionate use of low-dose Tacrolimus for 12 months in three patients with end-stage PAH increased their exercise capacity with amelioration of their functional class (FC), 6-minute walk distance (6MWD), N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, and augmentation of BMPR2 expression in the peripheral blood mononuclear cells (PBMCs).[50]

A phase IIa, placebo-controlled, double-blind, randomized, 16-week, single-center trial in 14 subjects with PAH, World Health Organization (WHO) functional class II/III symptoms was conducted using three Tacrolimus target levels (<2, 2–3 and 3–5 ng·mL-1) (NCT01647945).[51] Tacrolimus was generally well tolerated. While some subjects demonstrated a marked increase in BMPR2 expression, amelioration in 6MWD, and echocardiographic parameters, these changes were not significant.[51]

Chloroquine and Hydroxychloroquine

Chloroquine and hydroxychloroquine have shown favorable effects in preclinical animal PH models. The mechanism of action of chloroquine comprises inhibition of lysosomal degradation of BMPR2 as well as inhibition of autophagy pathways. Today, chloroquine is being used to treat malaria, as well as rheumatologic disease such as systemic lupus erythematosus (SLE).[52]

In preclinical studies, administration of chloroquine in monocrotaline (MCT)-exposed male Sprague Dawley rats was shown to prevent the elevation of pulmonary arterial pressures and RVH, to increase cardiac output, to inhibit the development of pulmonary artery remodeling, and to block the progression of established PH.[51] In rats cultured PASMCs, the administration of chloroquine was also shown to augment levels of BMPR2 expression, stimulate apoptosis and diminish PASMCs proliferation.[53]

The efficacy and safety of chloroquine has not been assessed in PAH clinical trials. The doses used in animal studies might be too elevated for humans.

Sotatercept

Disturbances in the bone morphogenetic protein receptor type II (BMPR2) and the transforming growth factor (TGF)-β pathways lead to cellular proliferation and vascular remodeling and cellular proliferation as seen in PAH. Sotatercept, a
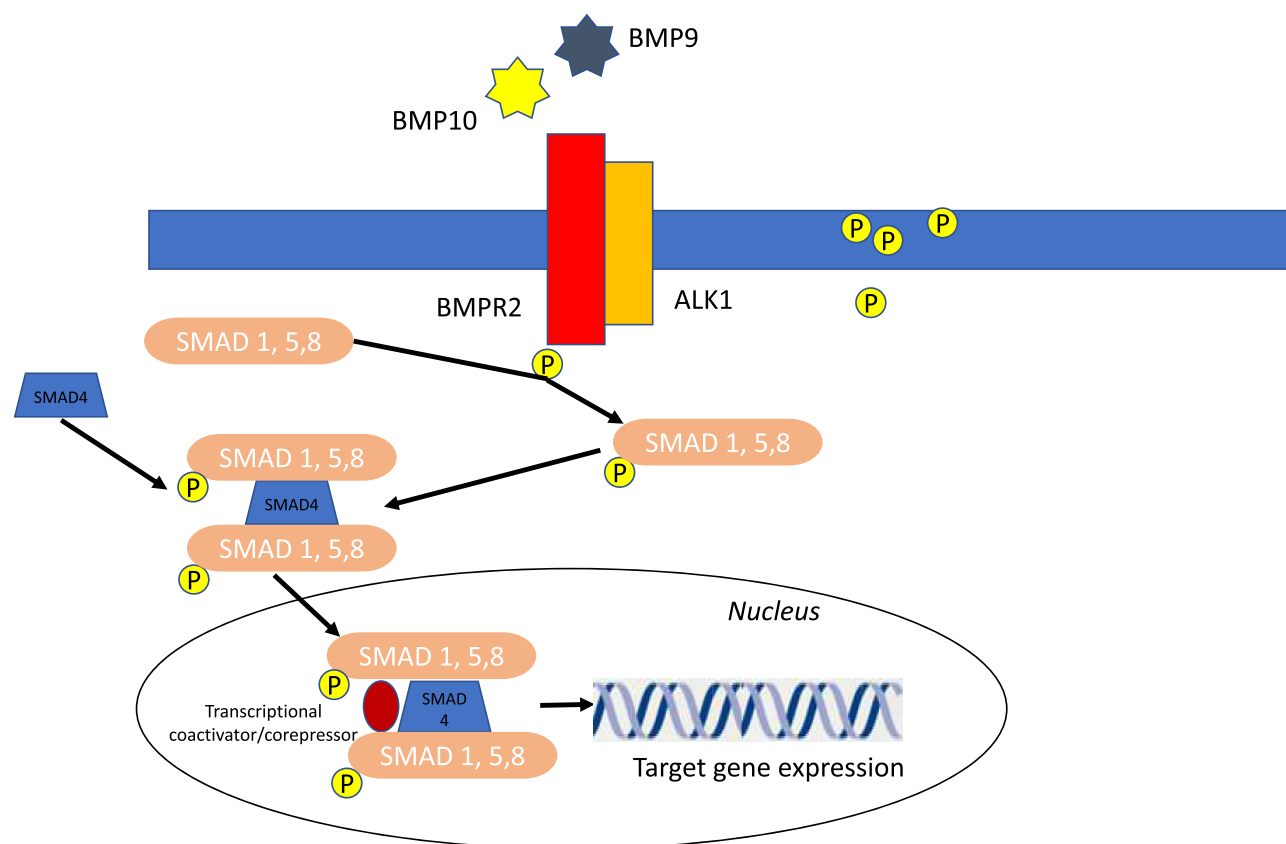
Figure 2 Schematic of BMP signaling pathway signaling implicated in PAH pathogenesis. BMP9 and BMP10 present in the circulation initiate signaling by binding and bringing together BMPR2 and ALK1. BMPR2 phosphorylated ALK1 which then propagate the signal through phosphorylation of SMAD 1/5/8. Subsequently, SMAD 4 forms a complex with SMAD 1/5/8, which translocates to the nucleus regulating the expression of target genes.
Abbreviations: ASK1, apoptosis signal regulating kinase 1; P, phosphate.

first-in-class, is a ligand trap with high selectivity for multiple proteins within the TGF-β superfamily, including activins and GDFs that can restore BMPR2 signaling and pulmonary vascular homeostasis. A Phase 2, multicenter trial with 106 PAH subjects showed that sotatercept in combination with background therapies for 24 weeks significantly reduces PVR and PA pressures, improves 6MWD, lessens NT-proBNP, and enhances WHO-FC compared with placebo.[54]

These results led to the STELLAR study, a Phase 3, randomized, double-blind, controlled trial that will evaluate the efficacy and safety of sotatercept 0.7 mg/kg SC every 3 weeks plus background therapy compared with placebo in 284 PAH patients. The primary end point of the trial is change from baseline in 6MWD. Secondary outcomes include hemodynamic improvements, time to death or clinical worsening, FC and QOL score changes (NCT04576988).

Inflammation and Immunity

Numerous PH experimental preclinical data and human studies have linked a malfunction of the inflammatory/ immune system to the development of PAH.[55] First, PAH is associated with systemic autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and scleroderma.[56–58] Second, perivascular inflammation has been frequently observed in subjects with idiopathic PAH[59] and PAH associated with systemic autoimmune diseases.[57,60] In lung sections of these PAH patients, perivascular lesions are characterized by the infiltration of immune cells of varying levels,[27] including B and T lymphocytes,[61–65] macrophages,[67] neutrophils,[66–68] dendritic cells,[65,69] and mast cells.[65,69] Third, higher levels of chemokines and cytokines such as tumor necrosis factor (TNF)-α, Interleukin-1 (IL-1), and Interleukin-6 (IL-6) are measured in PAH subjects.[27,70] These cytokines and chemokines include TNF-α, interleukin (IL)-1β, IL-6, IL-8, MCP-1 (monocyte chemoattractant protein-1), CCL5/ RANTES, fractalkine, and MCP-1 (monocyte chemoattractant protein-1). Some of these cytokines and chemokines are associated with a poor prognosis and might be used as markers of disease progression. Also, some, such

as IL-1β and TNF-α, have been linked to the pulmonary peri-vascular deposition of extracellular proteins such as fibronectin.[71,72] Others, such as IL-6, have been associated with the proliferation of PASMCs.[73] Fourth, in the MCT and chronic hypoxia rats models, anti-inflammatory agents have been shown to prevent and inhibit the development of PH.[74,75] Fifth, human studies have shown that the combination of immunosuppression and vasodilators may ameliorate pulmonary pressures, exercise capacity and prognosis of PAH subjects with certain systemic autoimmune diseases.[76,77] Finally, the absence of regulatory T cells (Treg), also known as suppressor T cells, the dysregulation of B cells, and the presence of endothelial autoantibodies have also been incriminated in the pathobiology of PAH. The lack of Tregs in athymic rats submitted to SU5416 and chronic hypoxia led to peri-vascular accumulation of B cells and development of anti-PAEC antibodies as well as severe PH.[78] On the other hand, the presence of Treg in euthymic rats was shown to inhibit the deposition of B-cells, development of anti-PAEC antibodies and elevation of PA pressures.[78]

The safety and efficiency of several agents modulating and regulating the immune system are currently being evaluated in clinical studies.

Therapies Targeting Inflammation and Immunity

Interleukin-6 (IL-6)

There is substantial evidence from the literature implicating elevated activity of the cytokine IL-6 in the development of PAH. PASMCs produce IL-6 as a pro-inflammatory cytokine.

In preclinical studies, the overexpression of IL-6 in mice was associated with elevated pulmonary pressures, RVH, and severe luminal obliteration of small pre-capillary arteries with infiltration of mast cells and lymphocytes.[79]

Hypoxia-induced PH transgenic IL6-deficient mice exhibit less inflammation and less pulmonary vascular remodeling compared to the wild-type mice.[80] IL-6 receptor (IL-6 R) was also shown to be up-regulated in PASMCs from PH experimental animal models leading to the development of pulmonary vascular remodeling with PAECs and PASMCs proliferation.[81] The administration of an IL-6R specific antagonist in experimental PH-induced rodent models prevented the development of PH.[81]

In human studies, IL-6 was shown to be augmented in serum and lungs of subjects with IPAH and PAH associated with connective tissue diseases (PAH-CTD). In PAH subjects, elevation of circulating IL-6 correlates with an increased risk of mortality.[82,83] Similar to the experimental animal models, IL-6R expression is upregulated in PASMCs from subjects with PAH.

Tocilizumab

Tocilizumab, also known as atlizumab, is an immunosuppressive drug given for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis. It is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R).

The significant improvement of several PAH-CTD subjects treated with tocilizimab[84–86] led to the Phase II TRANSFORM-UK clinical trial (Therapeutic Open-Label Study of Tocilizumab in the Treatment of Pulmonary Arterial Hypertension) (NCT02676947). This study was a 6-month open-label trial with tocilizumab in patients with group 1 PAH excluding those with SLE, RA, or CTD. Endpoints included safety change in exercise capacity, PVR and quality of life. Although the full data have not yet been reported, the drug was administered to 23 patients with a good safety profile. A larger, therapeutic study is expected.

Interleukin-1 (IL-1)

Serum levels of IL-1, which also promotes IL-6 synthesis, are augmented in PAH patients.[83]

In an inflammatory model of MCT-induced PH, increased IL-mRNA are expressed in the lung tissue[87] In the same animal model, the development of PH is prevented with administration of IL-1 receptor antagonist.[88] Furthermore, when IL-1 is administered to the R899X transgenic mouse with decreased BMPR2 expression, the rodent will develop significant pulmonary arterial remodeling with severely elevated pulmonary pressures.[89]

All these results are indicative of a strong association between IL-1 and inflammation in the PAH pathogenesis.

Anakinra

Anakinra is a recombinant of the IL-1 receptor antagonist and is approved for the treatment of RA.[90]

In an open-label study of 6 IPAH subjects, anakinra was shown to have a safe profile. (NCT03057028). In this small study, the six patients also had moderate functional impairment and right ventricular dysfunction at baseline.

After 2 weeks of treatment, a significant reduction of hsCRP with a trend reduction of IL-6 was observed. There was also substantial improvement in symptoms as assessed by the Minnesota Living with Heart Failure Questionnaire.[91] However, there was no significant change in circulating NT-proBNP, peak oxygen consumption, or RV systolic function compared to baseline.

This study indicates that anakinra in PAH appears safe with patients' symptomatology improvement. A longer and larger study is necessary to establish the efficacy of this agent in the treatment of group 1 PAH.

Tumor Necrotic Factor-α (TNF-α)

Systemic circulating TNF-α levels were shown to be more elevated in PAH patients compared to normal control subjects.[83]

In preclinical studies, transgenic mice overexpressing TNF-α will lead to the development of PH.[93] Also, in MCT-induced PH (MCT-PH) in dogs and rats, the lung expression of TNF-α is significantly augmented.[94,95]

TNF-α was shown to selectively reduce BMPR2 transcription and translation in PASMCs,[93] stimulate the pro-proliferative NOTCH2 pathway, and simultaneously inhibit the anti-proliferative NOTCH3 axis, leading to excessive PASMC proliferation.[92]

In animal models, the administration of anti- TNF-α agent was shown to improve the disease progression and to reestablish the normal BMP/NOTCH pathway.

These results confirmed the role of TNF-α in the pathogenesis of PAH, justifying the need of anti- TNF-α approaches in the treatment of PAH.[96]

Etanercept

Etanercept is a TNF-α inhibitor, which is approved to treat autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, juvenile idiopathic arthritis, psoriatic arthritis, plaque psoriasis, and ankylosing spondylitis.[97–100]

Etanercept was shown to prevent and reverse MCT-PH in rodents[92] and in endotoxemic pigs.[101] Etanercept in SU-5416 hypoxia rats restores BMPR2 expression, reduces NOTCH2 signaling activity, and normalizes activated SMAD function.[58] This leads to RVSP reduction, improvement of disease progression, RVH, and vascular remodeling.[96]

No study is currently investigating the safety and efficacy effect of etanercept in PAH patients. The above

findings suggest that a clinical trial should be considered in the future.

Nuclear Factor κB

Many of the inflammatory and immune pathways are under the control of the transcription nuclear factor κB. Nuclear factor κB stimulation leads to the upregulation of genes encoding IL-6, IL-1β, TNF, and other important immunologic signals.[102]

Multiple lines of evidence from rodent models have shown that nuclear factor κB blockade can prevent the development of PH.[103] IPAH subjects have activated nuclear factor κB in their lung tissue,[104] indicating a potential role for this pathway in human disease.

Bardoxolone

Bardoxolone methyl blocks nuclear factor κB by stimulating the regulatory transcription nuclear factor (erythroid-derived 2)–related factor 2,[105] activates Nrf2, and targets mitochondrial dysfunction.

In the phase II LARIAT trial (NCT 02036970) with 22 PAH subjects, administration of bardoxolone methyl vs placebo for 16 weeks showed a significant improvement in 6MWDT in the bardoxolone methyl group.[106]

A 24 week Phase III study with PAH-CTD patients (CATALYST) (NCT02657356), followed by a long-term open-label extension follow-up study (NCT03068130), with bardoxolone methyl vs placebo, to evaluate the change from baseline in 6MWD has been recently completed. Results have not yet been released.

Dimethyl Fumarate (DMF)

Dimethyl fumarate (DMF) activates the transcription factor nuclear factor erythroid-derived 2-related factor 2 (Nrf2) pathway and has been approved by the US Food and Drug Administration (FDA) as a treatment option for adults with relapsing multiple sclerosis.

DMF is considered to be an immunomodulatory agent, causing a shift in T helper cells (Th) and reducing inflammatory cytokine production, such as IL-6.

DMF improves pathological hemodynamics in PH-induced hypoxic mice models.[107] Not only does DMF prevent the development of PH and RVH in hypoxic and hypoxia/SU5416-treated mice, it also reverses PH in a chronic hypoxia mice model.[107] DMF blocks pro-inflammatory pathways, including NFκB, and STAT3 with evidence of less lung tissue infiltration by immune cells and

macrophages. Also, DMF was shown to inhibit HIF1α expression[108] and provide additional anti-PH effects.

A randomized, multicenter, double-blinded, placebo-controlled trial with DMF in 34 Systemic Sclerosis-PAH (SSc-PAH) patients is currently ongoing (NCT02981082). The primary outcome of this clinical trial is improvement in 6MWD. This study will also assess the safety profile of nuclear factor κB manipulation in patients with SSc-PAH.

Rituximab

Rituximab is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of immune system B cells.[109]

Following several case reports of disease regression in CTD-PAH with Rituximab, a randomized, Phase II, placebo-controlled, double-blind, NIH-funded ASC01 trial (NCT01086540) investigated the efficacy and safety of Rituximab on disease progression in patients with SSc-PAH with change in 6MWDT at 24 weeks as primary endpoint. Secondary endpoints included RV function changes evaluated by cardiac MRI. At week 24, 6MWWD trended towards improvement. Changes in PVR were highly variable but on average improved.[110]

Growth Factors and Tyrosine Kinases (TK) Signaling Pathway

Pathological proliferation of PAECs and PASMCs in PAH, as well as abnormal increase in the production of growth factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), have caused a paradigm shift in the treatment strategies of the disease as some researchers have associated the pathogenesis of PAH to a neoplastic process. These growth factors may act as powerful mitogens and chemo-attractants for SMCs, ECs and fibroblasts and may also cause resistance to apoptosis.[111]

PDGF can be synthesized in PAECs, PASMCs and macrophages and was shown to stimulate proliferation and migration of fibroblasts and PASMCs.[111] In PASMC and PAECs from lungs of PAH subjects, levels of PDGF and its receptor, PDGFR, were found to be elevated.[112] PDGF acts through the stimulation of two PDGF receptors (PDGFR-α and PDGFR-β), leading to fibroblasts and PASMCs' proliferation.

Increased PDGF signaling has been strongly implicated in playing an important role in the pathogenesis of PAH, including the efficacy of the PDGF pathway inhibitors in PH animal models. Animal model data highlight that

inhibiting the PDGF pathway may prevent/reverse the cellular proliferation of the intimal lining of the pulmonary arteries, a key characteristic of PAH.[113]

Tyrosine kinases (TKs) are enzymes that activate specific proteins by transferring a phosphate group from ATP to their tyrosine residues. Receptor tyrosine kinases (RTK) are composed of N-terminal extracellular ligand-binding domains and C-terminal intracellular tyrosine kinase domains which, upon ligand binding, catalyze phosphorylation of tyrosine residues of these RTKs, thereby altering their activity. PDGF acting via RTKs triggers the activation of major signaling pathways. RTKs have been associated with the pathogenesis of PAH.[114]

Different agents have been explored to block PDGFR and TK signaling pathway (Figure 3).

Therapies Targeting Growth Factors and Tyrosine Kinases (TK) Signaling Pathway

Imatinib

Imatinib is an oral chemotherapy agent used to treat chronic myeloid leukemia (CML),[115] gastrointestinal stromal tumors (GISTs)[116] and a number of other malignancies. Imatinib functions as a specific inhibitor of a number of tyrosine kinase enzymes. Imatinib inhibits PDGF-induced arterial remodeling and SMCs proliferation. In rat preclinical experiments, imatinib hinders PASMCs proliferation.[117]

Following reports of improvement in several PAH patients with Imatinib,[118,119] a phase II, multicenter trial evaluated the efficacy, safety, and tolerability of imatinib in 59 PAH subjects (NCT00477269). The study demonstrated that this agent was well tolerated. There was no significant change in 6MWDT, which was the primary end point. However, there was a significant decrease in PVR and an increase in cardiac output in imatinib-treated patients versus placebo.[119,120] Post hoc studies of this clinical trial showed improvement of 6MWD in a subgroup of patients with a PVR >1000 dyne·s·cm (−5),[120] indicating that imatinib might be efficient in severe PAH patients. These results led to the IMPRES trial (Imatinib in PAH, a Randomized Efficacy Study) (NCT00902174), which was a Phase III, 24-week, randomized, placebo-controlled, double-blind, multicenter clinical study evaluating the efficacy and safety of imatinib in 202 symptomatic PAH patients with PVR ≥ 800 dyne·s·cm (−5) on ≥2 specific PAH background therapies. At the completion of the study, the 6MWDT in the active treated group
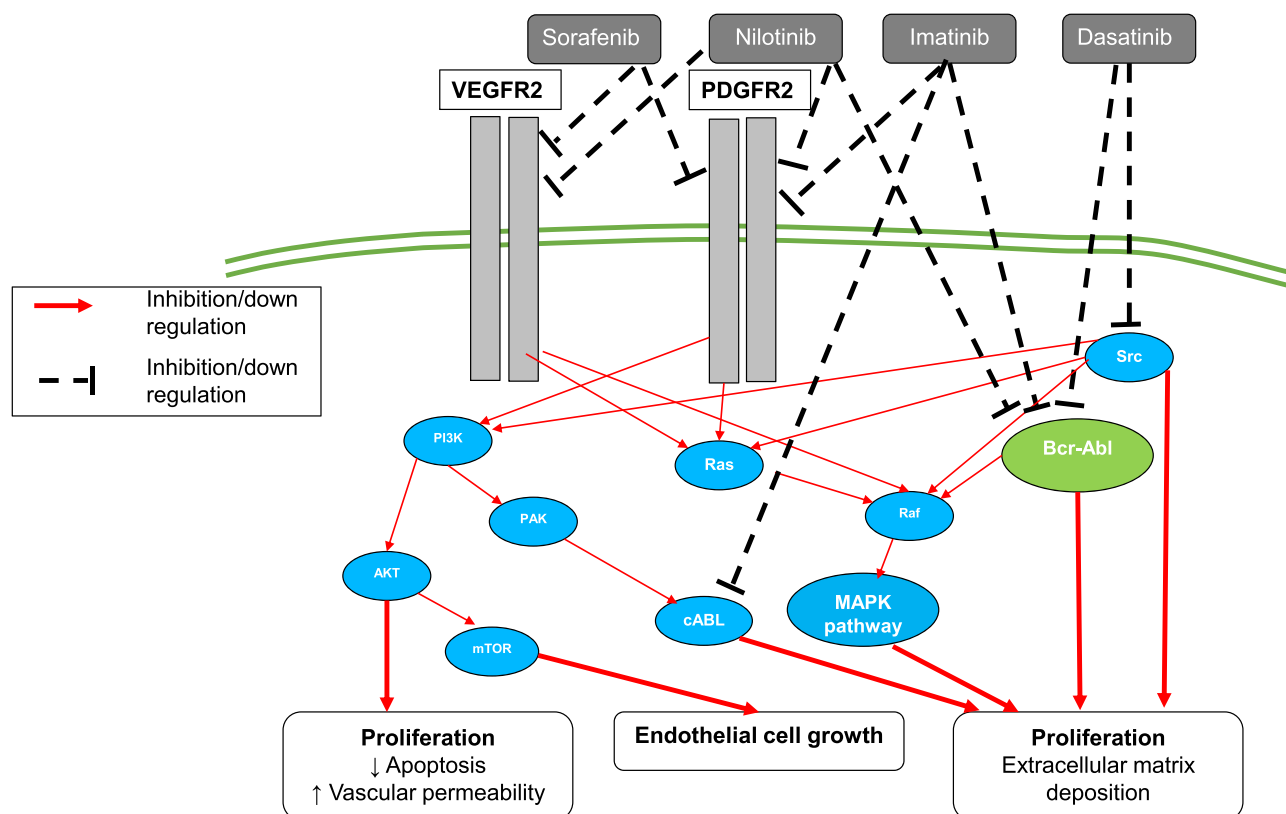
Figure 3 Growth factors and tyrosine kinases (TK) signaling pathway.
Abbreviations: ↑, increase in; ↓, decrease in; PDGFR2, platelet-derived growth factor receptor type 2; VEGFR2, vascular endothelial growth factor type 2; BCR, breakpoint cluster region; ABL, abelson 1 kinase.

improved by 32 meters compared to the placebo arm (*P*= 0.002), an effect maintained in the open-extension study. Also, the PVR significantly diminished by 379 dyne·s·cm (−5).[121] Moreover, treatment with imatinib was associated with significant improvement of the RV function compared to placebo in the echocardiographic IMPRES sub-study.[122] Nevertheless, discontinuations and serious adverse events were more common in the imatinib arm than in the placebo arm. Subdural hematoma occurred in eight patients who received both imatinib and anticoagulation.[121] The seriousness and the numbers of the adverse effects of imatinib arm, despite the strong efficacy of the drug, precluded the FDA approval of imatinib for the treatment of severe PAH patients.

Seralutinib

Although the studies of imatinib in PAH demonstrated significant hemodynamic and 6MWD improvements in PAH subjects, its poor tolerability and serious side effects suggested that while targeting the PDGFR and TK signaling pathway is significant as a potential therapeutic for PAH, a drug with an improved therapeutic window would be more optimal.

Based on this rationale, seralutinib was developed as a highly potent, small-molecule PDGFR kinase inhibitor that is formulated to be delivered via inhalation to limit systemic exposure and thereby improves tolerability and side effect profile of this class of drugs.

A current Phase II randomized trial with seralutinib is currently undergoing to determine the effect of seralutinib on improving pulmonary hemodynamics in subjects with PAH who are FC II and III with PVR improvement as primary endpoint. The secondary objective of this trial is to determine the effect of seralutinib on improving exercise capacity in this population. (NCT04456998)

Sorafenib

Sorafenib is multi-kinase inhibitor drug approved for the treatment of advanced renal cell carcinoma,[123] hepatocellular carcinoma,[124] FLT3-ITD-positive AML,[125] and radioactive iodine resistant advanced thyroid carcinoma.[126]

This tyrosine-kinase inhibitor also exerts activity against angiogenesis growth factor receptors PDGFR and VEGFR as well as against Raf-1 kinase, a regulator of endothelial apoptosis.[127]

Sorafenib demonstrated anti-pulmonary arterial remodeling effects and hemodynamic improvement in MCT induced PH rodent.[114,128] Sorafenib was shown to have positive hemodynamic effects in subjects with refractory PAH as well.[129]

A small exploratory phase Ib, single-arm open-label study, revealed that 16 weeks of sorafenib administration improved 6MWD significantly in 12 very limited PAH patients despite continuous administration of intravenous prostacyclin analog[130] (NCT00452218). Potential cardiovascular adverse effects with sorafenib have been described in several reports.

Nilotinib

Nilotinib, a second-generation TK inhibitor is structurally related to imatinib.[17] It is 10–30-fold more potent than imatinib in inhibiting BCR-ABL tyrosine kinase activity and proliferation of BCR-ABL expressing cells.[131–134]

Nilotinib is indicated for the treatment of both initial CML and CML resistant to imatinib as well as for the treatment of gastrointestinal stromal tumors.[135]

A Phase II multicenter, double-blind, randomized, 24-week, placebo controlled study was performed in PAH subjects to assess safety, tolerability and PK of nilotinib (NCT01179737). The trial was discontinued due to serious adverse reactions in the nilotinib arm. To be noted that nilotinib also carries a black box warning in the United States for possible heart complications.

Dasatinib

In contrast to imatinib, sorafenib, and nilotinib, dasatinib another second-generation BCR-ABL TK inhibitor indicated to treat positive CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL)[136] was shown in several reports to increase the risk of developing PAH.[137,138]

In October 2011, the US Food and Drug Administration (FDA) issued a safety announcement warning health care professionals of the elevated risk of developing PAH in patients receiving dasatinib. The drug is considered to induce lung vascular toxicity and predispose the development of PAH.

Thus, not all the TK inhibitors can be considered similar and may have potential opposite effects on PAH. Better comprehension of the different TK inhibitor properties and outcomes is needed.

RhoA/Rho-Kinase Inhibitors Signaling Pathway

Rho-kinase is part of a family of enzymes that are involved in regulating various cellular responses, such as cellular growth, contraction, gene expression migration, differentiation, (7) and, more specifically, smooth muscle tone.[139]

Intracellular signaling of the RhoA/Rho-kinase Inhibitor signaling pathway is increasingly appreciated as an important signaling pathway in the pathogenesis of PAH because of its vasoconstriction and its pro-proliferative effects of the pulmonary artery wall cells.[140,141]

Rho-A is a small GTP-binding protein that acts via its downstream effector Rho-kinase. This pathway couples membrane receptor/G protein signaling with phosphorylation of intracellular proteins. Rho-kinases contribute to agonist-induced vascular contraction via Ca^{2+} sensitization of PASMCs. Rho-kinase might also contribute to endothelial dysfunction through negative regulation of eNOS and phosphatidylinositol 3-kinase (PI3K) activity causing reduced endothelial NO bioavailability.

RhoA/Rho-kinase signaling pathway has been linked to various key upstream mediators (eg, endothelin, thromboxane, angiotensin II and serotonin)[142] (Figure 4).

Moreover, there is evidence that chronic hypoxia activates RhoA in PASMCs and PAEC.[143–146]

The importance of RhoA/Rho-kinase signaling in preclinical hypoxic PH studies is highlighted by the experiments of Fagan et al, in which the RhoA/Rho-kinase inhibitor Y267632, but not nifedipine, caused marked vasodilatation in hypoxic induced PH mice.[140]

In preclinical hypoxia-PH rodent studies, the RhoA/Rho signaling pathway showed vascular remodeling and vasoconstriction with sustained augmentation of PVR.[140,142]

Likewise, in humans, the RhoA/Rho kinase activity has been shown to be augmented in the PASMCs and PAECs of idiopathic PAH patients.[147]

Therapies Targeting Rho/ROCK Signaling Pathway: Rho-Kinase Inhibitors
Fasudil

Fasudil, a Rho-kinase inhibitor, has been studied in clinical trials as an antianginal[148] and for the treatment of cerebral vasospasm.[149]

In studies with MCT-induced PH rats, administration of Fasudil demonstrated improvement of PA pressures, arterial vascular remodeling and BNP levels compared to sildenafil and bosentan.[150] Clinically, fasudil administered intravenously has also shown significant improvement of hemodynamic parameters in subjects with severe PAH.[151]

A randomized, placebo-controlled, double-blind trial with 20 PAH subjects who received oral fasudil extended
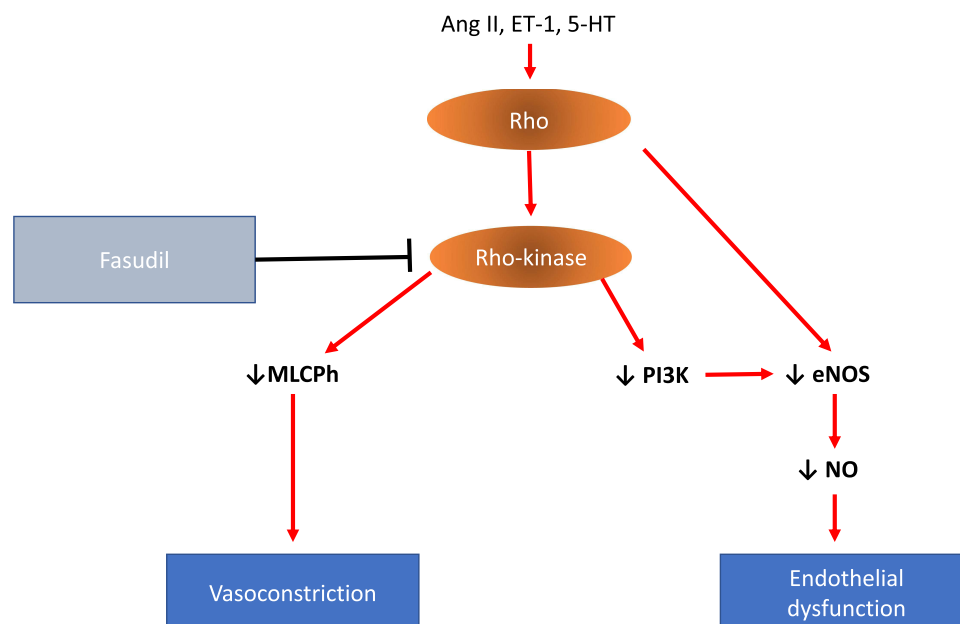
Figure 4 RhoA/Rho-kinase signaling pathway. Wthin PAEcs and PASMCs, Rho-Rho kinase can be activated by several pathological mediators, including angiotensin II (Ang II), endothelin-1 (ET-1) and 5-hydroxytryptamine (5-HT). Rho-Rho kinase activation leads to inhibition of myosin light chain phosphatase (MLCPh) and subsequently vasoconstriction. Rho-Rho kinase also contributes to endothelial dysfunction through negative regulation of endothelial nitric oxide synthase (eNOS) and phosphatidyli-nositol 3-kinase (PI3K) activity causing reduced NO bioavailability.
Abbreviations: ↓, decrease in; 5-HT, 5-hydroxytryptamine; Ang II, angiotensin II; ET-1, endothelin-1; eNOS, endothelial nitric oxide synthase; MLCPh, myosin light-chain phosphatase; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase.

release demonstrated a significant increase in the cardiac index after 30 months of fasudil administration; neverthe-less, no 6MWD amelioration was seen.[152] Preliminary results of another trial with fasudil demonstrated reverse pulmonary vascular remodeling effect.[153] Inhaled fasudil was also shown to lessen PVR significantly in a small PAH subject study.[154]

The positive experimental work with Rho-kinase inhi-bitors in both preclinical and clinical studies suggests the need for larger clinical trials to study their efficacy and safety in PAH.

Mitochondrial Dysfunction

Growing evidence has demonstrated the role of mitochon-drial dysfunction in the pathogenesis of PAH. Mitochondria control the redox-related enzymes, the proteins of the elec-tron transport chain, the regulators of proton gradient and apoptosis as well as calcium homeostasis and mitophagy. Mitochondria are the major source of energy production and are central to cellular metabolism. The metabolic path-ways of mitochondria comprise fatty acid oxidation, glucose oxidation, and glutaminolysis.[155–158] Mitochondrial dysfunc-tion in PAH is associated with increased production of acid lactic production and uncoupled glycolysis at the cost of pyruvate production and glucose oxidation.

Mitochondrial dysfunction might lead to pulmonary artery remodeling and therefore is linked to the underlying pathobiology contributing to the disease.[159]

Pulmonary vessels have a mitochondrial-metabolic phenotype comparable to that observed in cancer and described by Warburg.[160] This Warburg effect stimulates apoptosis resistance and proliferation of the pulmonary vessel cells.[10]

The Warburg effect mimics a pseudohypoxic state in which the transcription factor hypoxia inducible factor 1α (HIF-1α) is being upregulated. HIF-1α controls energy metabolism, vasomotor tone and angiogenesis. The HIF-1α upregulation takes place in spite of normal pO_2 and appropriately is called pseudohypoxia.

Increased HIF-1α is also caused by decreased levels of superoxide dismutase (SOD2) activity. The decrease in SOD2 activity found in PH will cause a reduction in the production of hydrogen peroxide (H2O2), generating this pseudo-hypoxia "milieu". The upregulation of HIF-1α inhibits the production of pyruvate dehydrogenase (PDH), leading to the PASMCs proliferation, apoptosis resistance and inflammation, which are key factors of the pathobiology of the disease and, finally, to the reduction of the acetyl-CoA entry into the Krebs cycle.[161]

Therapies Targeting Mitochondrial Dysfunction

Dichloroacetate (DCA)

The inhibitor dichloroacetate (DCA) was shown to activate pyruvate dehydrogenase, restore glucose oxidation, and prevent as well as reverse the development of PH in preclinical rodent studies.[162–164]

The effect of DCA to reverse the glycolytic shift, increase energy and ameliorate RV function in preclinical studies has led to an open-label, Phase I study to investigate DCA in subjects with PAH FC III–IV (NCT01083524).

In this 4-month, open-label study conducted in Canada and the UK, DCA was given to twenty PAH subjects already on standard PAH treatment and had a positive effect on symptoms. The treatment reduced PA pressures and PVR, improved lung metabolism as assessed by positron emission tomography (FDG-PET) scan, and ameliorated exercise functionality with a large variation of individual response.[165]

Selonsertib

Selonsertib (GS-4997), an inhibitor of apoptosis ASK1 (signal regulating kinase 1)[166] that also targets mitochondrial dysfunction, was evaluated in a Phase II study to determine the effect of selonsertib on PVR in 151 subjects with PAH over a period of 24 weeks (NCT02234141). Selonsertib did not significantly improve PVR compared to placebo.

Ranolazine

Ranolazine is a piperazine derivative that inhibits persistent or late inward sodium current (INa) in heart muscle[167] and promotes glucose oxidation in mitochondria. Ranolazine is currently approved for the treatment of chronic angina.[168,169]

In a rodent model of RVH, ranolazine was reported to successfully reverse metabolic dysfunction and improve cardiac output and exercise capacity.[170,171]

In a safety Phase I clinical trial (NCT01757808) 12 PAH patients were given ranolazine and were followed for 12 weeks. Ranolazine was found safe without significantly impacting hemodynamics (NCT01757808).[172]

Ranolazine was investigated in a 3-month phase III study with 11 PAH subjects (NCT01174173). In this study, ranolazine was shown to ameliorate RV function (p = 0.037), reduce RV size (p = 0.015), and enhance WHO FC (p = 0.0013) with a trend toward increase in exercise time on bike echocardiogram (p = 0.06). Nevertheless, no significant hemodynamics by right heart catheterization was observed.[173]

Another multicenter study (NCT01839110) looking at the effect of ranolazine in stable PAH patients with RV dysfunction (RVEF <45%) has been completed, but results have not yet been published.[174]

Trimetazidine

Trimetazidine is another piperazine derivative that also promotes glucose oxidation in mitochondria. Preclinical data have demonstrated that fatty acid oxidation inhibition by trimetazidine reverts the PH adverse phenotype by restoring mitochondrial function, decreases cellular proliferation and restores apoptosis susceptibility in PASMCs.

Preclinical rodent studies have demonstrated improvement of PA pressures and remodeling in animal treated with trimetazidine.[175]

A Phase II trial study is currently investigating the effect of trimetazidine on right ventricular function change in 25 PAH patients (NCT02102672).

Oxidative Stress

Abundant evidence suggests that oxidative stress (OS) is highly implicated in the pathobiology of PAH.

Increased levels of oxidative stress have been demonstrated in serum and lungs of animal PH models as well as PAH subjects.[176–179] Furthermore, the levels of oxidative stress increase concomitantly with the gravity of the disease, signifying that oxidative stress markers could potentially be used as markers of disease severity and evolution.[180–182]

Increased production of oxidant species and/or decreased production of antioxidants characterize oxidative stress. Oxidative stress is associated with augmented production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), diminished NO bioavailability and decreased glutathione peroxidase, superoxide dismutase (SOD), and catalase activity.[176]

ROS are created as a result of oxidation–reduction reactions and include free radical molecules such as superoxide ($O2-$), hydroxyl radical ($OH-$), lipid peroxyl and non-free radical species like hydrogen peroxide (H_2O_2). RNS like ($ONOO-$) are produced by the reaction between NO and $O2-$.[183] Cellular ROS are generated as by-products of mitochondrial respiration, NADPH oxidase, eNOS,[184] and xanthine oxidase.[185]

OS plays a crucial role in the pathobiology of PH[176,186–188] as it causes vessel wall thickening by stimulating the activity of transforming growth factor-β1 (TGF-β1), VEGF,

fibroblast growth factor-2 (FGF-2),[189] and PDGF.[190] Additionally, OS can trigger vasoconstriction by increasing the production of endothelin-1[191,192] and thromboxane A2,[193] and by decreasing prostacyclin levels.[194,195] Also, OS was shown to upregulate the transcription of the factors HIF-1α and HIF-2α,[196,197] factors that are also involved in the development of PAH.[198,199] Finally, augmented OS causes inflammation and cell damage via oxidation of proteins, lipids and DNA.[184,186,200–203]

Preclinical rodent models have demonstrated that increased OS contributes to the pathogenesis and the development of PH.

Extracellular superoxide dismutase (SOD3) is essential for removing extracellular superoxide anions and is highly expressed in the lungs. SOD3 appears to play a major role in protecting against the development of PH through decreasing O_2^- and increasing H_2O_2 and NO bioavailability. SOD3 knockout mice and SOD3 loss of function gene mutation in rats resulted in the development of PH, RVH and vascular remodeling in response to hypoxia or MCT.[204]

Therapies Targeting Oxidative Stress

In spite of a wealth of data demonstrating the involvement of the oxidative stress in PH, and several cases reports of antioxidant therapies improving PH in animal models, these agents have been essentially ineffective in treating PAH subjects.

Dihydroartemisinin (DHA)

Dihydroartemisinin (DHA) is an anti-inflammatory, anti-malaria, and anti-tumor agent. DHA blocks PAECs proliferation and diminishes OS by augmenting SOD expression and decreasing ROS in MCT-PH rats.[205]

No clinical studies have yet been performed to investigate the effects of DHA in PAH subjects.

Fenofibrate

The PPARα agonist fenofibrate is another anti-oxidative stress therapy. In MCT-PH rats, fenofibrate reduces RVH, OS, ROS, and NADPH oxidase (NOX-1) activity.[206] No clinical trial has yet investigated the effects of fenofibrate in PAH patients.

Trapidil

Trapidil is a potent vasodilator, which was shown to improve the effects of OS in PH in augmenting lipid and glutathione peroxidation as well as decrease RV dilatation in MCT-PH experimental animals.[207]

No clinical studies have been conducted to investigate the effects of trapidil on PAH subjects.

Nitrite

NO_2^- is a reactive nitrogen species (RNS) created by the oxidation of NO. In animal models of PH, nebulized nitrite caused reduction in mean PAP and regression of pulmonary remodeling.[208] Also, administration of inorganic nitrate, which is metabolized into nitrite and NO, was shown to improve the severity of PH in mice.[209]

However, a phase II trial single center evaluating the effect of inhaled nitrite in 48 PAH subjects undergoing RHC did not significantly improve PVR (NCT01431313).

Coenzyme Q

Coenzyme Q (CoQ) is an antioxidant via the redox cycle. CoQ participates in oxidative phosphorylation and plays a critical role in biochemical generation of ATP.

In a prospective randomized double-blind study with 15 PAH patients evaluating the use of the coenzyme Q10 vs placebo for 12 weeks, there was a small enhancement in right ventricular hemodynamics with no significant improvement in 6MWD or biomarker levels (NCT01148836).[210]

Allopurinol

Xanthine oxidase (XO) generates oxygen species. XO catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid with the creation of four superoxide anions.[211] Thus, XO plays a key role as a regulator of the cellular OS.[212]

Under conditions of tissue hypoxia in an experimental model,[213] the breakdown of ATP to AMP to hypoxanthine provides substrate to XO. Subsequently, XO uses oxygen rather than NAD as an oxidant. As a result, XO produces superoxide and hydrogen peroxide (H_2O_2) rather than NADH.[214,215] Increased vascular $O_2^{\bullet-}$ production due to XO affects negatively endothelial function by impairing nitric oxide (NO) signaling[216] and triggering pulmonary arterial remodeling.[217]

Rodent animal studies have tested the effect of allopurinol in PH.

Elevated levels of serum and lung xanthine oxidase activity as well as increased levels of phosphatidylcholine hydroperoxide, a marker of oxidative stress have been demonstrated in rats exposed to chronic hypoxia[217,221] Administration of allopurinol to these hypoxic rats decreased PCOOH levels, improved pulmonary vascular remodeling, and diminished RVH.[217,218]

In PAH clinical studies, an abnormal role for the xanthine oxidase pathway was also demonstrated. In a study with 99 IPAH subjects, there was a strong association between the right atrial elevation and the level of uric acid.[219] In another retrospective study with 29 PAH patients, uric acid levels correlated with New York Heart Association class. Also, elevated uric acid levels correlated with lower 6MWD and a worse survival.[220] Additionally, xanthine oxidase activity is increased in subjects with PAH compared to normal controls.[221] Moreover, expression of the antioxidant enzyme SOD2 is reduced in PAH lungs.[186,222]

Despite the potential benefits of treating PAH patients with allopurinol, no clinical study has investigated the potential benefits of allopurinol in PAH subjects.

Extracellular Matrix (ECM)

The extracellular matrix (ECM) plays a critical role in controlling cell shape and cell signaling, in maintaining cell–cell communication, and in regulating cell differentiation. Vascular stiffness is directly associated with the proportion of the different ECM components regulating PASMCs contractility and proliferation.[223–225] Elastin and collagen are the main structural ECM constituents. A variety of growth factors and cytokines regulate the production of the various components of the ECM. Turnover of the ECM is controlled by the balance between proteolytic enzymes such as serine elastases and matrix metalloproteinases (MMPs). The MMPs are matrix-degrading enzymes implicated in ECM turnover and in PASMCs migration and proliferation. It is, therefore, not surprising that perturbations in the regulation of the ECM would lead to pathogenic pulmonary vascular remodeling and PAH.

Remodeling of the extracellular matrix (ECM) with augmented collagen deposition and cross-linkage as well as increase in the elastic laminae breakdown are characteristics of PAH.[19]

Among the MMPs, MMP-2, and MMP-9 are the major MMPs in the pulmonary vessels. They are implicated in the PASMC stimulation and neointimal development characterizing the vascular remodeling seen in PAH.[226,227] MMPs are regulated by the tissue inhibitors of metalloproteinase (TIMP) TIMP-1 and TIMP-2. The imbalance of MMPs/TIMPs will lead to matrix abnormality and remodeling.[228] Also, pulmonary arterial stiffness stimulates proliferation of PASMCs through mechano-activation of several signaling pathways, including transcriptional

cofactors YAP/TAZ, transforming growth factor-β, Toll-like receptor, and NF-κB.[229]

Preclinical studies have revealed that transgenic expression of MMP-9 is augmented in the monocrotaline-PH rodent model. The data further point out that MMP-9 contributes to the fibrosis and remodeling of pulmonary vessels in this experimental model. Inhibition of MMP-9 should be considered as possible PAH treatment.[230]

Therapies Targeting the Extracellular Matrix
MMP Inhibitors

Preclinical research has revealed potential therapeutic benefits with MMP inhibitors in the monocrotaline-PH rodent preclinical studies.[231,232]

A study demonstrated that intratracheal instillation of human TIMP-1 gene in the lungs of rodents subjected to monocrotaline improved pulmonary vascular remodeling, RVH, and muscularization of small pulmonary vessels.[233]

Several pharmacological compounds have been shown to improve PH via regulation of MMP/TIMPs.[234]

Lercanidipine, a vasoselective dihydropyridine calcium channel blocker, showed improvement in PAH subjects by reducing serum MMP-9 levels and OS with no modification of proMMP-2 activity or TIMP-1 level.[235,236]

The calcium channel blocker, amlodipine, administered to the MCT-PH rat was shown to inhibit MMP-2 activity, platelet activation, PAECs damage, and PASMCs proliferation.[237]

The endothelin receptor antagonist, bosentan, was also shown to significantly diminish MMP-2, TIMP-1, and NO synthase activity in monocrotaline-induced PH rodent.[238]

Periostat, the only MMP inhibitor currently approved by the FDA, is only indicated for periodontal disease. The probable cause for its low use in clinical practice is its numerous undesirable adverse reactions and poor oral bioavailability.

Serine Elastase Inhibitor: Elafin

The neutrophil elastase, which is be secreted by PASMCs, plays a fundamental role in the adverse remodeling of the ECM of the pulmonary vessels in PAH.

Transgenic mice overexpressing elafin, a serine elastase inhibitor when subjected to hypoxia, showed diminished serine elastase and MMP activity compared to controls. Elafin transgenic mice demonstrated improved PH pressures, reduced muscularization and conservation

of the peripheral small pulmonary arteries compared to the non-transgenic mice.[239]

Although the clinical use of elastase inhibitors has been limited by hepatotoxicity, the endogenous elastase inhibitor. Elafin, remains a promising therapeutic possibility. Elafin, in addition to blocking the action of elastase, enhances BMPR2-mediated signaling, inhibits nuclear factor κB and diminishes the innate immune system.[240] Administration of elafin in animal models reverses PH.[232] As such, clinical exploration of elafin as a PAH therapy is underway, beginning with a safety and tolerance trial in healthy volunteers (NCT03522935).

Metabolism
Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ)

Emerging evidence has demonstrated that the Peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway dysregulation is involved in PAH development.[241–244] PPAR-γ, a nuclear transcription factor, is known to modulate various cellular and physiological processes including cell differentiation, lipid metabolism, inflammation, and tumorigenesis.

In healthy PAECs and PASMCs, PPAR-γ is profusely expressed, but its expression declines significantly in the PAECs and PASMCs of PH affected patients and animals.[242]

Moreover, the elimination of PPAR-γ from PAECs and PASMCs produces PH and activation of PPAR-γ reduces the PH development.[245] PPARγ plays a key role in regulating the BMP2/BMPR2 and transforming growth factor–β1 (TGFβ1) pathways in vascular SMC, regulating several miRNAs, and cell proliferation, as well as the glucose metabolism.[246,247]

PPAR-γ acts downstream of BMP-RII. BMP2/BMPR2 activates PPARγ in PASMC,[16] and mRNA expression of both BMP2[248] and PPARγ[242] is decreased in lung tissue from IPAH patients.

Thus, PPAR-γ has been proposed to be an important therapeutic target for PAH.

Therapies Targeting PPAR-γ

A class of drug substances that are known to be potent agonists of PPAR-γ are thiazolidinediones. Rosiglitazone and pioglitazone, for example, are two thiazolidinedione-based orally active drugs used in type 2 diabetes. These drugs improve glycemic control and enhance insulin sensitivity in diabetic patients by selectively activating PPAR-γ in the liver, skeletal muscle, and adipose tissue.

Rosiglitazone

Rosiglitazone has been shown to decrease PASMC proliferation by modulating cell growth and apoptosis.[37,249] Rosiglitazone reduces mPAP in both MCT- and chronic hypoxia-induced PH animal models.[250,251] Unlike other anti-PH drugs, which reduce only the pulmonary pressures but have little or no direct effect on vascular remodeling, rosiglitazone appears to reduce pulmonary pressures and pulmonary vascular remodeling in PH animals.[249]

At the molecular level, rosiglitazone stimulates adiponectin, which suppresses PDGF with subsequent inhibition of PASMC proliferation.[252] In chronically hypoxic rats, rosiglitazone increases PPAR-γ levels and decreases endothelin (ET1) and VEGF levels.[253] In hypoxic mice, rosiglitazone reduces PH by improving oxidative signaling by decreasing superoxide generation and PDGF activation.[251]

Pioglitazone

In monocrotaline-PH rats, pioglitazone was shown to improve pulmonary pressures, as well as peripheral pulmonary vessel muscularization and wall thickness. Furthermore, RVH and fibrosis are also attenuated.[254]

In SU-5416 hypoxia rats, pioglitazone decreases PA pressures, reduces pulmonary vascular remodeling and improves RV function with reduction of RV fibrosis.[255] Mitochondrial function and organization from right ventricular cells also improve. Pioglitazone appears to stimulate multiple genes involved in the FAO pathway, with subsequent utilization enhancement of fatty acid. Finally, pioglitazone was shown to reduce the elevated levels of miR-197 and miR-146b in SU-5416 of the RV cardiomyocytes.[255]

Although the preclinical data are promising, the administration of pioglitazone and rosiglitazone for PAH patients should be considered carefully as these drugs can cause fluid retention and HF.[256]

No clinical studies have been conducted to investigate the effects of rosiglitazone and pioglitazone in PAH subjects.

Metformin

In experimental studies, metformin, a biguanide agent used in type 2 diabetes mellitus,[257] has demonstrated favorable effects on the prostacyclin, endothelin and nitric oxide signaling pathways. Metformin, via AMPK activation, inhibits

the proliferation of murine PASMCs induced by ET-1.[258] AMPK activation also causes an increase in NO synthesis via stimulation of eNOS.[259] Furthermore, Metformin increases the tyrosine nitration of prostacyclin synthase.[260] Additionally, Metformin was shown to block the estrogen pathway via inhibition of aromatase transcription,[261] and to decrease PASMCs stimulation by blocking MAPK (mitogen-activated protein kinase)[262] and by decreasing Rho kinase activity.[262] In MCT rats, administration of metformin reduces pulmonary vascular remodeling, decreases pulmonary pressures and RVH.[262]

Metformin in PAH subjects is presently being tested in a phase II clinical study (NCT03617458). The primary end points include safety and measure of OS biomarkers. Secondary end points include BMPR2 expression in mononuclear cells, RVEF and RV volume measures using MRI, and 6MWD.

Neurohormonal Modulation

In PAH, both sympathetic nervous (SNS) and renin–angiotensin–aldosterone (RAA) systems are activated. Even though agents targeting the sympathetic or RAA signaling pathways have been shown to improve PH in animal models, the efficiency of such treatments in humans has been ambiguous.

Sympathetic Nervous System Activation

A large body of evidence has confirmed the contribution of the sympathetic nervous system in the pathogenesis PAH.[263,264]

In one study with 60 group 1 PAH subjects, circulating norepinephrine levels inversely correlated with cardiac output.[265] In another study with 32 PAH patients, circulating norepinephrine levels strongly correlated with PVR and 5-year mortality rate.[266]

Similarly to the left ventricle in HFrEF, the right ventricle in PAH, undergoes maladaptation with downregulation and desensitization of α-1 and β-1 adrenergic receptors.[267,268]

Therapies Targeting Sympathetic Nervous System Activation

Preclinical animal studies have demonstrated that β-blockade improves pulmonary arterial remodeling. For example, arotinolol, an α- and β- adrenergic receptor blocker, reduces pulmonary arterial pressures in MCT rats.[269] Propranolol and nebivolol were both shown to stimulate NO activity by blocking the protein kinase C.[270,271] Nebivolol also demonstrated improvement of the pulmonary vascular remodeling in PH-rodent models.[272]

Carvedilol demonstrated improvement in survival as well as matrix deposition, RVH, and Nrf2 pathway in MCT-treated animals.[273,274]

Administration of bisoprolol to MCT-rats demonstrated an increase in RV contractility and cardiac output as well as a decrease in the degree of inflammation and fibrosis.[275]

A few small clinical trials have investigated the efficiency and safety of β-blockade in PAH.

In an open-label trial with 6 PAH subjects, the use of carvedilol increased RV systolic function by cardiac MRI.[276]

In a double-blind, placebo-controlled, randomized study with 30 PAH subjects (NCT01586156), carvedilol was shown to increase RV function at 3 but not 6 months and did not improve exercise capacity or cardiac output. In this study, carvedilol was also shown to increased β-adrenergic receptor density on white blood cells.[277] In another placebo-controlled, crossover, randomized, single-center study with 18 IPAH patients (NCT01246037), no benefit with bisoprolol was seen, with even a drop in cardiac index, along with a trend towards a decreased in 6MWD. RVEF also remained unchanged.[278]

Renin–Angiotensin–Aldosterone System (RAAS)

Activation of the RAAS in PAH is well recognized.[279–282] Even if benefits from ACE inhibitors and angiotensin II receptor antagonists have been well demonstrated in HFrEF, their positive effect cannot be extrapolated to the field of pulmonary hypertension. Clinical trials with ACE-inhibitors in PAH have demonstrated no benefit.[283,284]

Although AT-1 receptor antagonism has demonstrated benefit in the MCT rat model of PH, this drug class has not been trialed in humans with PAH.[279]

ACE2 is a homologue of ACE that is insensitive to ACE inhibitors. It converts angiotensin I and angiotensin II to angiotensin-(1–7), angiotensin-(1–9), and angiotensin-(1–5). These vascular peptides have both cardiac and vascular protecting effects. Ang-(1–7) activates the Mas receptor (Mas1), which is present on ECs and has vasodilatory, anti-inflammatory and anti-fibrotic effects,[285,286] functionally antagonizing the effects of AT1 receptor stimulation.[287–289]

Low ACE2 levels and antibodies against ACE2 have been measured in the blood of PAH subjects and may potentially play a role in the pathogenesis of PAH.[290]

Therapies Targeting Renin–Angiotensin–Aldosterone System

A small open-label study with 5 PAH subjects demonstrated that one infusion of rhACE2 (purified intravenous formulation of soluble recombinant human ACE 2) was associated with improvement of PA pressures and reduction of inflammatory and OS biomarkers.[291] However, another open-label, dose-escalation study with a single dose of rhACE2 was not able to confirm the significant improvement of the PVR (NCT03177603).

Aldosterone Antagonist

Aldosterone is a mineralocorticoid hormone that plays a central role in the regulation of the circulatory homeostasis including blood pressure, sodium level, and potassium retention. Aldosterone is implicated in the development of myocardial fibrosis and stimulation of the adrenergic autonomous system.[292] Mineralocorticoid receptors are located in numerous organs including the pulmonary vasculature.

Current clinical indications of mineralocorticoid-receptor antagonists include treatment of HFrEF[293] and refractory ascites in cirrhotic patients.[294]

In preclinical studies, aldosterone levels are elevated in the serum and lungs of PH-MCT rodents.[296] Similarly, in PAH patients, serum aldosterone levels are increased and their levels correlate with PVR.[295]

In PH-induced rodents, elevated serum aldosterone was shown to reduce eNOS in PAECs,[296] promote maladaptive vascular remodeling including fibrosis,[297] and stimulate PASMC proliferation in the pulmonary vasculature.[298] Additionally, aldosterone negatively regulates endothelin type B receptor.[296]

Clinical studies in PAH subjects with a mineralocorticoid receptor antagonist such as spironolactone have demonstrated conflicting results.

A single-center, 16-week randomized double-blinded crossover clinical trial was performed to assess the effect of spironolactone (50 mg daily) in 42 patients with PAH. At the end of week 8, the treatment arm was switched. The treatment with spironolactone was safe but no change in the levels of amino-terminal propeptide of procollagen type III (PIIINP), MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio at weeks 8 and 16 was seen compared to baseline and placebo arm. No change in walk distance was noted at weeks 8 and 16.[299]

A post-hoc study from ARIES 1 and ARIES 2 clinical trials indicated some positive effects in the addition of spironolatone to ambrisantan.[300] Subsequently, a prospective study examined in 30 subjects with PAH, the effect of spironolactone when added to ambrisentan on exercise capacity (NCT02253394). Unfortunately, due to low enrollment, the study was terminated early.

Another randomized, double-blind, multicenter, placebo-controlled study (NCT01712620) is currently investigating the long-term effects of spironolactone in 70 subjects with PAH.

Vasoactive Factors

Vasoconstriction is one of the vascular changes that have been identified in PAH, implicating an imbalance between vasodilator and vasoconstrictor factors. Several vasoactive factors that may affect pulmonary vascular tone, including natriuretic peptides, adrenomedullin, vasoactive intestinal peptide (VIP) and relaxin, have been studied in PAH.

Natriuretic Peptides

Natriuretic peptides (atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and c-type natriuretic peptide) activate soluble guanylate cyclase, and increase intracellular cGMP production via stimulation of soluble guanylate cyclase, with subsequent vasodilation and PASMCs anti-proliferation effect. Also, the natriuretic peptides relax vascular SMCs, inhibit salt and fluid retention, block PASMCs proliferation and endothelin-1 synthesis, and antagonize many of the actions of the renin–angiotensin–aldosterone system. Circulating levels of ANP and BNP correlate directly with the degree of RV failure. ANP and BNP bind to natriuretic peptide receptor-A and B.[301–303]

All three natriuretic peptides have been shown to inhibit hypertrophy of cardiac myocytes in vitro.[304] Mice with transgenic overexpression of ANP have smaller RVs than their non-transgenic littermates.[305]

ANP and BNP are potent pulmonary vasodilators[306–310] and have been shown to blunt hypoxia-induced PH and RVH in preclinical rodent animal models[311,312] Also, the natriuretic peptides may play an important role in limiting RVH during the development of PAH.

One mechanism by which BNP limits its anti-PAH effects may be by its profound inhibitory effect on TGF-β-induced pro-inflammatory, fibrotic, and proliferative effects on cardiac fibroblasts.[313]

Nesiritide

Nesiritide is a recombinant form of BNP and is FDA approved for the management of acute decompensated

HF. Nesiritide ameliorates hemodynamic variables, such as the pulmonary capillary wedge pressure, in patients hospitalized for HF management.[314–316]

The infusion of IV nesiritide was shown to ameliorate pulmonary pressures and wedge pressures in 10 subjects with PH secondary to LV dysfunction (post-capillary PH). However, the drug did not demonstrate hemodynamic improvement of the other 10 patients with PAH (pre-capillary PH).[317]

Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP), is produced by PAECs and is a potent systemic vasodilator. VIP decreases PA pressure and PVR in MCT-PH rabbits[318] and in healthy human subjects.[319] VIP inhibits PASMCs proliferation[320] and platelet activation.[321] Also, PAH has been associated with low levels of VIP in the serum and PAH lungs.[322]

In a preliminary case series, eight patients with PAH treated with inhaled VIP showed marked clinical and hemodynamic improvement.[322] However, a double-blinded, phase II, randomized trial with 56 PAH receiving inhaled VIP was negative.[323] The reasons for these discrepant findings are unclear and may be due to the dosing or delivery system.

Another randomized, multicenter, double-blind phase II clinical trial investigating PB1046, a VIP analogue, is currently being conducted (NCT 03556020).

Adrenomedullin

Adrenomedullin vasodilates pulmonary vessels, increases pulmonary blood flow, and is synthesized in the normal lung by several cell populations. High levels of mRNA for adrenomedullin and its receptor in the lungs suggest a regulatory role for the peptide in the pulmonary circulation.[324]

Plasma levels of adrenomedullin are elevated in preclinical PH animal models as well as in PAH patients.[325–327] Plasma levels of adrenomedullin have been correlated with CVP, PVR and mean PA pressure, suggesting that levels of adrenomedullin increase in proportion to the extent of PAH.[328]

Administration of adrenomedullin improves PA pressures as well as medial thickening of pulmonary arteries in MCT-rats.[329] Similarly, the infusion of adrenomedullin to PH subjects was shown to improve PVR.[330–332] Nonetheless, larger and longer studies in human PAH with adrenomedullin are needed to examine its efficacy and long-term effects.

Relaxin

The anti-fibrotic, anti-inflammatory and vasodilatory parturition natural peptide hormone, relaxin,[333] was shown to cause positive hemodynamic effects in an experimental PH rat model.[334] Clinically, relaxin in PAH subjects is elevated compared to normal controls.[335] No clinical trial with relaxin in PAH subjects is currently ongoing despite its potential therapeutic effect.

Serotonin System

Serotonin (5-hydroxytryptamine or 5-HT) is a vasoconstrictor that promotes PASMC hyperplasia hypertrophy and proliferation.[336]

Serotonin is a potent growth factor released from the endothelium.[337] It has a paracrine effect on neighboring cells and diffuse to the vascular SMC layer.[337] In the normal vasculature, serotonin is largely stored in circulating platelets in the bloodstream.[338] This sequestration helps to maintain low normal plasma levels of 5-HT.[338] Increased 5 HT levels in the plasma and low platelet storage are observed in PAH.[339] 5-HT is mitogenic.[340] Also, the 5-HT pathway elicits endothelial-dependent and PASMCs vasoconstriction.[341] The 5 HT-induced growth and proliferative effect appear to be dependent on the RhoA/ROCK pathways.[342,343]

Elevated plasma serotonin levels and reduced content of serotonin in platelet have been reported in patients with IPAH.[344]

Use of appetite suppressant medications such as dexfenfluramine, fenfluramine, aminorex, and benfluorex, which increase platelet serotonin release and inhibit its re-uptake, is associated with an increased incidence of PAH.[345]

Data have described elevated expression of the 5HT transporter (5-HTT) in the PASMCs.[346,347] Mutation in the 5HTT and/or 5-HT receptors in the platelets and lung tissues from PAH patients has also been demonstrated.[346]

5HT2A/2B Receptor Antagonists

Terguride

Terguride is a serotonin (5-HT2A and 5-HT2B) receptor blocker.[348–350]

In the experimental MCT-PH rodent model, the administration of terguride was shown to inhibit the development of PH and RVH.[351] Nonetheless, a clinical phase II, placebo-controlled, randomized trial revealed no hemodynamic or clinical beneficial effects. Also, significant side effects were reported in the terguride group.[352]

Selective Serotonin Reuptake Inhibitors (SSRIs)

The SSRI, escitalopram was tested in PAH subjects in a clinical trial, but results were never released (NCT00190333). Another phase II, 3-month, open-label trial examining the tolerability and safety of fluoxetine was performed with PAH subjects, but results were never published (NCT 00942708).

Estrogens Signaling Pathway

Regulation of the estrogen signaling pathway may also lead to novel potential PAH therapies.[353] Indeed, the estrogen metabolism pathway and its interaction with other PAH signaling pathways appear to be PAH disease modifiers. The focus of the estrogen signaling pathways in the understanding of PAH pathogenesis is based on the prevalence of the disease affecting women more than men with a female/male ratio of 3–4:1.[354–357] Furthermore, estrogen was shown to downregulate the expression of BMPR2 in the pulmonary vasculature.[358]

Data have found that 17β-estradiol (E2), the most common female sex hormone and its metabolite, 16α-hydroxyestrone, are important factors in the development of PAH.[353,359] Higher circulating E2 levels were measured in PAH-men compared to normal controls in a case–control study.[360] E2 is mostly synthesized through aromatisation of androgens by the enzyme aromatase (encoded by the *CYP19A1* gene).

In hypoxia-induced PH rodents, estrogen enhances RV function and administration of 17b-estradiol improves exercise endurance, probably by inhibition of RV myocytes apoptosis and anti-inflammation mechanism.[361] Furthermore, estrogen also improves the right ventricle by protecting mitochondrial organization, structure, and oxidative function.[362]

It is puzzling that while women are more susceptible to develop PAH than men, they exhibit better RV function and survival than men with PAH.[363,364] It is also interesting to note that women with PAH seem to respond better to specific PAH treatment than their male counterparts.[363]

Finally, PAH postmenopausal women and PAH-men were found to have more elevated circulating levels of estrogen with lower levels of dehydroepiandrosterone sulfate (DHEA-S) than female and male controls.[360,365] Elevated levels of estrogen are associated with worse 6-minute walk distances while elevated levels of DHEA-S were linked with lower right atrial pressure and PVR.

Therapies Targeting the Estrogen Signaling Pathway

Anastrozole

Anastrozole is an antiestrogen agent used in women in the treatment and prevention of estrogen receptor-positive breast cancer. Anastrozole blocks the enzyme aromatase, which catalyzes the conversion of androgens to estrogens.[366,367]

In experimental PH models, the positive effects of anastrozole are seen in female rodents but not in males.

Female mice exposed to chronic hypoxia and then treated with anastrozole have diminished vessel remodeling, less elevated RV systolic pressure (RVSP), and less hypertrophy.[368] Their counterpart hypoxic PH-male, on the other hand, does not exhibit any beneficial effect from the administration of anastrozole.[368]

In another protocol with SU-5416 hypoxia rats, and again only in females, anastrozole, decreases the amount of remodeled vessels.[368] The difference in gender response might be explained by lesser amounts of aromatase activity in PASMCs in males than in females. Indeed, males have less aromatase activity in the SMCs of their pulmonary arteries than the female rodents.[368]

The use of the combination of anastrozole and fulvestrant, another selective estrogen receptor modulator, to obtain complete estrogen blockade formation was shown to inhibit pulmonary arterial remodeling and lessen the pulmonary pressures in the BMPR2 R899X transgenic female rodent model.[358]

Clinically, anastrozole was shown to significantly decrease 17b-estradiol levels by 40% and improve the 6MWD by 26 meters in a 12-weeks randomized clinical trial with 18 PAH patients (NCT01545336).[369] There was, however, no change in quality of life or RV function.[369] Based on these results, PHANTOM (Pulmonary Hypertension and Anastrozole Trial) a phase II, NIH-funded, randomized, double-blind, placebo-controlled multicenter trial, is currently assessing safety and 6MWDT change in 84 PAH patients with anastrozole administered for 12 months (NCT03229499).

Tamoxifen

The effect of tamoxifen, another selective estrogen receptor inhibitor,[370] is also being studied. In this randomized, double-blind, single-center, placebo-controlled trial, tolerance and impact of tamoxifen on functionality, biomarkers, echocardiographic parameters, 6MWDT, and quality

of life for 24 weeks in 24 PAH patients will be evaluated (NCT03528902).

Several other NIH-funded trials targeting the estrogen and androgens signaling pathways in PAH, including studies with fulvestrant (NCT02911844), tamoxifen (NCT03528902), and DHEA (NCT03648385)(NIH HL141268) are currently ongoing. Results are not yet available.

Iron Deficiency

In PAH, a large body of evidence has demonstrated the importance of iron metabolism. In several PAH registries, iron deficiency has been shown to be common,[371–374] is being associated with diminished functional capacity[372,373] and survival.[375] Furthermore, iron deficiency in PAH is associated with disease severity including higher pulmonary pressures, diminished cardiac output and worse functional class.[340] Positive effects of iron administration in PAH patients have been shown in two small clinical trials,[376,377] even though another study has demonstrated that a low iron level might be protective.[378]

A multicenter phase II trial with 40 PAH subjects examined the effect of intravenous Ferinject® (ferric carboxymaltose) on functional capacity and PVR (NCT01447628). Results are not yet published. Another 120week, open-label trial also studied the effects of oral ferrous sulfate in 40 PAH (NCT01446848). No results for this trial have been reported either.

Vitamin D Deficiency

Recent data have established the consequences of vitamin D deficiency in PAH. In a 68 PAH patients study compared to 100 controls, vitamin D deficiency was shown to be highly prevalent. Also, 25-hydroxy vitamin D levels appear to be a potential predictor of adverse outcomes in PAH.[379] In this study, 70% of the PAH studied subjects had severe vitamin D deficiency with secondary hyperparathyroidism. PAH subjects with total 25-hydroxy vitamin D plasma above the median (7.17 ng/mL) exhibited better 6MWDT, exercise capacity, functional class, RV systolic function and survival. Lower levels of bioavailable 25-hydroxy vitamin D were linked to lower exercise capacity, more advanced functional class, and higher risk of mortality.[379]

In a rat model with PH, administration of 1,25 $(OH)_2D_3$, the bioactive metabolite of vitamin D, was shown to restore normal lung function by modifying the function of miR-204 through the transforming growth factor (Tgfbr2), TGFβ-1 and Smad pathways.[380]

Restoration of vitamin D levels in the PAH population represents a very achievable goal. Whether symptoms, prognosis and survival of PAH subjects improve after restoring vitamin D levels remains unclear. In a uncontrolled small cohort of PAH subjects, restoring vitamin D levels for 3 months was shown to ameliorate 6MWD and right-ventricular size without improving the pulmonary pressures or the functional class.[381] Thus, a large randomized study is warranted to evaluate the long-term effects of vitamin D substitution in the PAH population.

DNA Damage

Reports have shown that in patients taking etoposide, an anticancer drug causing DNA damage and PARP-1 activation may develop PAH.[382] Poly [ADP-ribose] polymerase 1 (PARP-1), member of the PARP family, detects DNA damage and contributes to the repair of DNA by modifying the structure of the chromatin and by interacting with multiple DNA repair factors.[383]

As discussed previously, PAH is characterized by sustained inflammation and elevated circulating cytokines such as TNF-α and IL-6,6 both of which are known to promote DNA damage.[384–386]

Increased DNA damage in the distal pulmonary arteries in PAH has been demonstrated in comparison with control patients. Also, PARP1 activity was shown to be augmented in PASMCs from subjects with PAH.[387] In PAH, PARP-1 appears to promote the activation of a pro-proliferative and anti-apoptotic program, via miR-204 downregulation and subsequent NFAT and HIF-1 (hypoxia-inducible factor 1-α) upregulation in PASMCs.[387]

It is possible that PARP-1 activation is a common denominator for diverse conditions that cause DNA damage in PAH, making it an attractive therapeutic target.

Therapies Targeting DNA Damage
Olaparib

PARP-1 inhibition is associated with a complete reversal of miR-204 downregulation, limiting NFAT activation, and decrease in PAH-PASMC proliferation.[387] In preclinical studies, inhibition of PARP-1 reduces PH severity in MCT and SU-5416 hypoxia rodents.[387]

Olaparib, an orally available PARP1 inhibitor approved for the treatment of BRCA (breast cancer gene)-related breast cancer, is being investigated in a small clinical trial

(NCT03782818). Twenty PAH subjects are getting Olaparib for 24 weeks, with PVR change, as primary end point.

FoxO1 Signaling Pathway

FoxO1 is a transcription factor that belongs to the forkhead O family and plays a critical role in biological processes, including inflammation, metabolism, proliferation, and vascular homeostasis.[388,389] FoxO1 regulates PASMC stimulation via its pro-apoptotic properties.[390]

In MCT and SU-5416 hypoxia rats as well as in PAH patient, FoxO1 is downregulated and inactivated.[390,391] This change will cause PASMCs stimulation leading to the development of PH.[390]

Therapies Targeting FoxO1 Signaling Pathway
Paclitaxel

Paclitaxel is one of the most potent and effective antineoplastic agents and is widely used in a variety of tumors including breast, ovarian, and lung cancers.[392] Paclitaxel activates cellular apoptotic program by inhibiting microtubule depolymerization and arresting cycling cells in M (mitotic) phase.[393]

Paclitaxel was shown to stimulate FOXO1. Activated FOXO1 reduces cellular proliferation and promotes apoptosis by modifying the expression of numerous cell-cycle modulators and by stimulating the BMPR2 pathway.[390,394]

In both MCT and SU-5416 hypoxic rats, administration of paclitaxel activates BMPR2 signaling and induces PASMCs apoptosis resulting in lesser PH, increase in RV systolic function and decrease in RV dilatation and hypertrophy.[390,395]

Paclitaxel is currently not being tested in clinical trials, probably due to its potential numerous adverse effects.[396] Use of an inhaled form of the drug should, nevertheless, be considered as it was shown to be effective in animal models with limited systemic effects.[390]

Emerging Therapies

Over the last decade, vast research efforts have led to the development of inventive therapeutic approaches in the field of PAH. Undeniably, recent preclinical studies indicate that immunotherapy, gene therapy, microRNA-based therapy and epigenetic medicines may offer new therapeutic perspectives in the area of PAH.

Immunotherapy

Therapeutic vaccine is a new approach for the treatment of oncologic and cardiovascular diseases. Vaccines generate antibodies against specific target molecules and may offer a new safe and effective way to treat PAH.

Activation of the endothelin system and more specifically of the endothelin-1 receptor type A (ETAR) have been implicated in the pathogenesis of PAH.[397] ETRQβ-002 is a vaccine developed against the second extracellular loop (ECL2) of ETAR and was administered to MCT-rats and Sugen/hypoxia-exposed mice. The ETRQβ-002 vaccine was shown to significantly decrease RV systolic pressures and improve pathological remodeling of pulmonary arterioles in both the MCT-rats and Sugen/hypoxia-induced mice, two and 3 months after the vaccine injection.[398] The results of this positive study may provide a novel approach for the treatment of PAH.

Gene Therapy

Abnormal or malfunctioning genes due to mutation or abnormal gene expression may promote the development of PAH by stimulating PASMCs and PAECs proliferation, causing apoptosis resistance and by altering the integrity of PAECs.[399]

Heritable PAH (hPAH) represents approximately 6–10% of all PAH. Genetic studies have identified more than 450 mutations heterozygous germline mutations in the *BMPR2* gene.[400]

Gene therapy is a technique that focuses on the utilization of the therapeutic delivery of nucleic acids into a patient's cells to treat disease.[401] Thus, delivery of targeted-gene such as *BMPR2* may treat, block the disease progression or even prevent the development of PAH. The delivery of the adenoviral vector comprising the *BMPR2* gene was shown to improve PH, RVH and pulmonary vessel remodeling in the chronic hypoxia-PH rodent model.[402]

The use of the adenovirus vectors remains limited due to its strong inflammatory reaction associated with its gene transfer.[403] Advances in gene delivery technology have led to the development of new vectors with limited inflammatory reaction called adeno-associated virus (AAV). The use of such vectors is currently being used in experimental PH models.[404]

MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are small, non-coding endogenous RNA molecules consisting of 17–25 nucleotides.[405,406] A

single miRNA can regulate hundreds of genes or proteins and conversely multiple miRNAs can regulate one protein. miRNAs target both mRNA degradation and suppression of protein translation on the basis of sequence complementarity between miRNA and its target.

Currently, many miRNA have been discovered and found to have a critical role in the pathogenesis of many disease, including PAH.[407]

Several miRNAs expressed in human lungs with PAH have been found to be altered compared with normal controls. While miR-138, miR-145 and miR-210 are upregulated in PASMCs of PAH subjects,[408–410] miR-124, miR-204, and miR-206 are downregulated.[411–413] Also miR-204 down-regulation appears to be strongly associated with PAH severity and seems to correlate with PASMCs proliferation and PAECs apoptosis resistance levels.[412]

Also, intratracheal nebulization of miR-204 was shown to improve pulmonary vascular remodeling and RVH in a MCT-PH rat experimental model.[412]

Epigenetics

Growing data indicate that epigenetics plays a crucial role in the development of PAH. Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence.[414] Examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence. Gene expression can be controlled through the action of repressor proteins that attach to silencer regions of the DNA.

The discovery of epigenetic components to PAH may offer new potential therapeutic targets for the treatment of PAH.

DNA Methyltransferase (DNMT) Inhibitors

DNA methylation is the modification of DNA nucleotides by addition of a methyl group. Because DNA methylation affects cellular function through successive generations of cells without changing the underlying DNA sequence, demethylating agents are considered a type of epigenetic therapy. Demethylating agents block the activity of DNA methyltransferase (DNA methyltransferase inhibitors/DNMT inhibitors). Currently, two members of this class, azacitidine and decitabine, are FDA-approved for use in the United States in myelodysplastic syndrome and are being investigated for use in a number of tumors.[415]

The activity and expression of the mitochondrial superoxide dismutase-2 (SOD2) have been shown to be significantly diminished in PAH subjects and PH rodent experimental models. No mutation was found in the *SOD2* gene in PAH patient and rat's PH experimental models. However, study of the *SOD2* gene has revealed selective hypermethylation of the SOD2 promoter region in fawn-hooded rats, known to develop PH spontaneously.[3] Administration of the DNMT inhibitor methyltransferase 5-aza-2′-deoxycytidine was shown to re-establish SOD2 expression, block cell proliferation and apoptosis resistance in the fawn-hooded rats' PASMCs.[156] These results indicate that targeting DNA methylation can be an appealing therapeutic possibility to restore SOD2 expression in PAH.

No clinical study has yet been performed to investigate the effects of 5-aza-2′-deoxycytidine in PAH subjects.

Histone Deacetylase (HDAC) Inhibitors

Histone deacetylases (HDAC) are enzymes that eliminate the acetyl group from histone proteins, altering the chromatin condensing process and approachability of transcription factors. HDAC inhibitors represent a new class of cytostatic agents that inhibit the proliferation of tumor cells in culture and in vivo by inducing cell differentiation, cell cycle arrest, and/or apoptosis. HDAC inhibitors exert their anti-tumor effects via the induction of expression changes of oncogenes or tumor suppressor, through modulating the acetylation/deacetylation of histones and/or non-histone proteins such as transcription factors.[416]

Preclinical experiments demonstrated that HDAC1, and HDAC5 are upregulated in PAH patients as well as experimental animal models of PH.[417] Both valproic acid, a class I HDAC inhibitor, and suberoylanilide hydroxamic acid (vorinostat), an inhibitor of class I, II, and IV HDACs, were shown to lessen the development and at least to partially reverse rat hypoxia-induced PH.[417]

Likewise, the activity of the transcription factor myocyte enhancer factor 2 (MEF2) was demonstrated to be significantly decreased in PAECs of PAH subjects. MEF2 regulates the expression of miRNA-424 and miRNA-523, two miRNAs involved in the maintenance of pulmonary vascular homeostasis[418] as well as other number of transcriptional targets involved in pulmonary vascular homeostasis, such as microRNAs 424 and 503, connexins 37 and 40, and Krüppel-like Factors 2 and 4, which were found to be significantly diminished in PAH PAECs. The impaired MEF2 activity in PAH PAECs was found to be

linked to excessive accumulation of HDAC4 and HDAC5 that block MEF2 function. The selective inhibition by MC1568 of class IIa HDACs led to restoration of MEF2 activity in PAECs by inducing degradation of HDAC4 and HDAC5 in both the MCT and the Sugen animal models with subsequent significant decrease in cell proliferation in the pulmonary vasculature.[419]

Overall, these studies validate the therapeutic potential of targeting histone acetylation using HDAC inhibitors to inhibit vascular remodeling in PAH.

Bromodomain and Extra-Terminal Motif (BET) Inhibitors

Epigenetic mechanisms include the placing (writing) or removal (erasing) of histone alterations that permit the chromatin to change to an open, activable chromatin state necessary for transcription. A third, less studied epigenetic pathway includes the reading of these specific histone marks once placed. The BETs (bromodomain and extra-terminal-containing protein family), which includes BRD2, BRD3, BRD4 and the testis-restricted BRDT, are epigenetic "reader" regulator proteins. They bind to specific acetylated lysine residues on histone tails and modulate gene transcription involved in many cell functions, such as cell proliferation and apoptosis.[420] The BETs facilitate the assembly of transcription complexes including transcription factors like RNA Polymerase II. BRD4 is the most characterized studied member of the BET family. Compared to controls, BRD4 was shown to be upregulated in lungs, distal PAs, and PASMCs of PAH patients. BRD4 expression in PAH is microRNA-204 dependent.[421]

BRD4 inhibition, by JQ1, was shown to increase p21 expression, and trigger cell cycle arrest. Moreover, in the same study, blocking BRD4 inhibition by JQ1, demonstrated a decrease in the expression of three major oncogenes, which are overexpressed in PAH, survivin, the nuclear factor of activated T cells, and the B-cell lymphoma 2. Inhibition of these oncogenes leads to diminished PAH-PASMC proliferation. BRD4 inhibition also significantly improved hemodynamic measurements by significantly decreasing mean PA pressures and by increasing cardiac output in the Sugen/hypoxia-induced PAH rat model. Finally, BRD4 inhibition by JQ1 was shown to decrease pulmonary artery wall area by 40%.[421]

These promising preclinical results led to the ongoing early Phase 1 pilot study assessing apabetalone in the PAH population (NCT03655704). Apabetalone (development code RVX-208) is an orally available small molecule that targets bromodomain and extra terminal domain (BET) proteins, and in particular BRD4. The overall hypothesis of this pilot study is that BRD4 inhibition with apabetalone is a safe and effective therapy for PAH to assess the feasibility of a Phase 2 clinical trial.

Conclusions

The hallmark of PAH is a lethal and progressive hyperproliferative vascular remodeling process that involves all cell types of the pulmonary artery. Initially thought to be a disease primarily characterized by vasoconstriction, it is now recognized that the pathobiology of PAH is much more complex and includes cellular apoptosis resistance, genetic abnormalities, altered microRNA function, increased extracellular matrix deposition with enhanced interaction interplay of inflammation, metabolic derangements, and mitochondrial dysfunction, leading to pulmonary artery remodeling and elevated pulmonary vascular resistance.

Although major advances in our understanding of the mechanism of disease development and treatment have been achieved, substantial gaps in our knowledge remain.

Current approved PAH therapies do not cure the disease and appear to have limited effects on the underlying pathogenesis.

While multiple novel signaling pathways in the pathogenesis of PAH have been discovered over the last three decades, most clinical trial investigating new therapeutic agents targeting these pathways so far have been disappointing. Hence, the importance and necessity to improve comprehension of the PAH pathobiology and to discover new molecules capable of disrupting the disease and its development as well as improving outcomes of PAH.

Disclosure

Dr Zolty is a consultant at Actelion, Bayer, United Therapeutics, and Alnylam. The author report no other conflicts of interest in this work.

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