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## New and Emerging Therapies for Pulmonary Arterial Hypertension

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### Abstract

Pulmonary arterial hypertension (PAH) is a pulmonary vasculopathy that causes right ventricular dysfunction and exercise limitation and progresses to death. New findings from translational studies have suggested alternative pathways for treatment. These avenues include sex hormones, genetic abnormalities and DNA damage, elastase inhibition, metabolic dysfunction, cellular therapies, and anti-inflammatory approaches. Both novel and repurposed compounds with rationale from preclinical experimental models and human cells are now in clinical trials in patients with PAH. Findings from these studies will elucidate the pathobiology of PAH and may result in clinically important improvements in outcome.

### Keywords

hypertension; pulmonary; right heart failure; clinical trials; humans; pulmonary arterial treatment

## INTRODUCTION

Pulmonary hypertension is defined by elevated pulmonary arterial pressure (PAP). Pulmonary arterial hypertension (PAH), one of five categories delineated by the World Health Organization (WHO), is characterized by pulmonary vascular remodeling in the small pulmonary arteries, increased PAP, and elevated pulmonary vascular resistance (PVR) that is not attributable to other heart, lung, and sleep disorders. PAH itself has several forms, which share similar histopathologic findings, including intimal proliferation, medial smooth muscle cell (SMC) hypertrophy, and adventitial thickening. PAH can be idiopathic (not associated with known genetic mutations or other comorbidities), heritable (with a significant family history or documented genetic mutation), or associated with connective tissue disease (CTD-PAH), drugs and toxins (including anorexigens, such as fenfluramine,

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### DISCLOSURE STATEMENT

E.S. is an inventor on a Stanford University patent, No. 6148B17, "Use of FK506 for the treatment of pulmonary arterial hypertension," and is a Scientific Advisor to Seltin Pharma, LLC, and Vivus Inc.

and recreational drugs, such as methamphetamine), portal hypertension (termed portopulmonary hypertension), HIV infection, or congenital heart disease.

The prevalence of all forms of PAH is approximately 15–50 per million individuals with an incidence of 2.4 per million per year (1). For many types of PAH (idiopathic, heritable, portopulmonary), female sex is a risk factor; up to 80% of adult patients in some series are women. Although outcomes among the types of PAH vary, incident patients with PAH have an ~15% risk of death within one year and a 30% risk of death within three years.

Symptoms are progressive, including shortness of breath, fatigue, chest discomfort, abdominal fullness, and lightheadedness or syncope with severe disease. These symptoms and the high risk of death are attributed to increases in PVR and reduced vascular compliance, resulting in increased right ventricular (RV) afterload and progressive RV dysfunction. RV morphologic changes and RV failure portend worse outcomes in PAH.

There has been dramatic progress in treating PAH over the past 20 years. Fourteen US Food and Drug Administration (FDA)–approved therapies delivered via oral, inhaled, subcutaneous, and intravenous routes target three established mechanistic pathways of PAH, which are endothelin, prostacyclin, and nitric oxide (NO) signaling. Endothelin receptor antagonists, prostacyclin analogues or receptor agonists, and phosphodiesterase 5 inhibitors and a soluble guanylate cyclase activator improve exercise capacity, and some treatments prolong time to adverse clinical endpoints in PAH. For patients who worsen despite these therapies, bilateral lung or heart–lung transplants are the final treatment options; however, there are strict criteria for candidacy and transplant is a complex surgical procedure with its own set of risks.

Despite major advances in the understanding of and treatment for PAH and the introduction of multiple approved therapies, most patients with PAH will still die from their disease, and there is no cure. Investigators have therefore pursued novel therapeutic targets on the basis of recent discoveries surrounding the pathophysiology of PAH. This review focuses on new mechanistic pathways with interventions that have advanced beyond the preclinical stage in patients with PAH.

## SEX HORMONES

### Preclinical Studies

The historical and early modern reports of individuals with PAH almost universally described women. More recent registries and cohort studies from the United States and other countries have confirmed that women have a higher risk of PAH (mainly idiopathic, heritable, and portopulmonary) than men do. The past decade has seen a surge of interest in the role of sex in experimental and clinical PAH, although the term estrogen paradox has been used to summarize the sometimes-incongruous findings (see Reference 2). For example, only females of certain animal models develop pulmonary hypertension (serotonin transporter overexpression, S100A4/Mts1 transgenic, and dexfenfluramine). Other animal models [monocrotaline (MCT), hypoxic rat, endothelial nitric oxide synthase (eNOS), vasoactive intestinal peptide, and apolipoprotein E knockout] show less severe pulmonary

vascular disease in females than in males. In addition, administration of estrogen is protective in certain animal models (decreasing pulmonary vascular remodeling and improving RV dysfunction), whereas estrogen actually worsens the pulmonary vascular changes in other models. Finally, testosterone increases RV hypertrophy and worsens RV function in the pulmonary artery banding model of pulmonary hypertension, suggesting that estrogen is not the only hormone of interest (3).

Estrogen downregulates expression of *BMPR2*, the gene that codes for bone morphogenetic protein receptor type II [a transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily receptor], mutations in which cause heritable PAH, and affects miR-29 (4, 5). Genetic variants in cytochrome P450 (*CYP1B1*) increase the risk of PAH in *BMPR2* mutation carriers by shifting estrogen breakdown toward pro-proliferative, antiapoptotic metabolites (6). MacLean and colleagues have shown the mechanistic importance of aromatase and *CYP1B1* in the MCT and Sugen-hypoxia models of PAH (where they are found in the small muscular pulmonary arteries themselves) as anastrozole and metformin (both of which inhibit aromatase) reduce the number of remodeled vessels and lower PAP (7, 8). Similarly, administration of anastrozole and fulvestrant (an estrogen receptor blocker) reversed pulmonary vascular remodeling in the *BMPR2* transgenic mouse model (9).

### Clinical Studies

Many of the findings in experimental models, which are inspired by clinical observations, are directly translatable to humans. Adult women without clinical cardiovascular disease have better RV ejection fraction (RVEF) than men do (10). Higher estrogen levels in women using hormone therapy are associated with RVEF (11); genetic variants in estrogen signaling and androgen signaling are associated with RV structure and function in women and men, respectively (12). Whereas women have a higher risk of PAH than men, women with PAH have better RV function and better survival than men (13, 14); these findings are potentially attributable to a better RV response to treatment in women than in men (13). Last, men and postmenopausal women with PAH have higher levels of estrogen and lower levels of dehydroepiandrosterone sulfate (DHEA-S) compared with male and female controls (15, 16). Higher levels of estrogen are linked with shorter six-minute walk distance (6MWD), whereas higher DHEA-S was associated with lower PVR and right atrial pressure.

These data have led to clinical trials of treatments targeting the sex hormone profile. One small placebo-controlled randomized clinical trial (RCT) of anastrozole showed a reduction in circulating estrogen levels and no change in tricuspid annular plane systolic excursion (a marker of RV function) (primary endpoints) but a significant increase in 6MWD over 12 weeks (17). A larger National Institutes of Health (NIH)-funded phase II RCT of anastrozole in PAH is currently enrolling (NCT03229499). Several federally funded trials are targeting estrogen or androgens in PAH, including studies of fulvestrant (NCT02911844), tamoxifen (NCT03528902), and DHEA (NIH HL141268).

## GENES, EPIGENETICS, AND miRs

### Preclinical Studies

More than 70% of patients with familial/heritable PAH and 20% of patients with idiopathic PAH have heterozygous mutations in *BMPR2* (18). Less frequent gene mutations in PAH belong to the BMP pathway (18), and other forms of PAH also exhibit dysfunctional BMP signaling, making PAH treatments targeting this pathway promising in general.

Experimental approaches to restore BMPR2 signaling include exogenous *BMPR2* delivery by gene therapy, mutation correction through drugs that facilitate nonsense-mediated decay (NMD) read-through (i.e., Ataluren) (19), improvement in BMPR2 trafficking to the membrane by chemical chaperons (4-sodium phenyl-butyrate, hydroxychloroquine) (20), inhibition of lysosomal degradation (i.e., SMURF-1 inhibition, elafin) (21, 22), delivery of exogenous ligand (e.g., BMP9) (23), increases in *BMPR2* expression (e.g., paclitaxel) (24), or Tgf-b1/3 ligand trap (25). In a high-throughput screen of FDA-approved drugs, Spiekerkoetter et al. (26) identified the immunosuppressive drug FK506 (tacrolimus) as an effective BMPR2 signaling activator. Low-dose FK506 improved endothelial dysfunction and reversed experimental pulmonary hypertension in the MCT and Sugen-hypoxia rat models by increasing BMPR2 signaling by removing the TGF- $\beta$  pathway inhibitor FKBP12 as well as by inhibiting calcineurin (26).

As small noncoding RNA molecules, miRNAs regulate gene expression and impact on pulmonary vascular development, physiology and disease, BMPR2 regulation, metabolism and proliferation, DNA damage, estrogen signaling, and vasoconstriction (27).

Dysregulation of miRNAs is integral to events that shape the development and progression of PAH (28). Whereas some miRNAs have been described in a specific cell and functional context (29), others have been identified by means of computational network modeling as “master regulators” of multiple genes that are important in PAH (30, 31). Possible miRNA therapies include inhibitors of miR-17, miR-130/301, miR-143/145, miR-20a, and miR210 and mimics of miR-204, miR424/503, and miR-96 (27); the data are insufficient to support one of these targets over the others at this point. In addition, strategies to deliver miRNA mimics or antagomirs to patients are still in their infancy. Important issues that need to be addressed include the route of delivery, the mode of delivery (naked oligonucleotides, via vectors, packaged in nanoparticles), and the potential for off-target effects.

DNA damage and abnormal DNA repair lead to a pro-proliferative, cancer-like phenotype underlying the progressive pulmonary vasculopathy of PAH (32–34). DNA damage in peripheral blood mononuclear cells, pulmonary arterial endothelial cells, and SMCs predates clinical PAH, as healthy family members and PAH patients with different etiologies (idiopathic PAH, heritable PAH, and disease-associated PAH) demonstrated susceptibility to DNA damage and mutagens before disease was apparent (35). Furthermore, DNA damage can be induced by reactive oxygen species, inflammation, hypoxia, reduced BMPR2 expression, anorexigen drugs, and (meth)amphetamines (32, 36); this damage persists for years after the injurious insult, suggesting an additional mechanism for PAH even after removal of the triggering exposure.

DNA damage induces poly (ADP-ribose) polymerase (PARP-1), which is responsible for DNA repair. However, overactivation of PARP-1 is seen in PAH and leads to cell dysfunction and activation of an inflammatory response. Genetic deletion or pharmacological inhibition of PARP-1 protected against endothelial dysfunction, vascular remodeling, and elevated RV pressure as well as RV hypertrophy (34, 37).

### Clinical Studies

FK506 has been the only drug tested in a clinical trial to increase BMPR2 signaling. FK506 was employed for compassionate use in three advanced PAH patients on maximal medical therapy who were listed for lung transplantation (38). All three patients stabilized after one year of low-dose FK506 treatment (trough level 1.5–2.5 ng/ml) in terms of symptoms, 6MWD, NT-proBNP, and RV function. A 16-week, placebo-controlled RCT included 23 stable PAH patients and showed that FK506 (blood level 1–5 ng/ml) was well tolerated (39). Although the study was not intended to assess efficacy, some patients (targeting blood levels of 3–5 ng/ml) had a significant increase in BMPR2 expression in peripheral blood mononuclear cells, supporting further studies. Olaparib, an oral PARP-1 inhibitor that is FDA-approved for ovarian cancer, is being studied in an open-label single-arm study with the primary endpoint of change in the PVR over 16 weeks (NCT03251872).

## ELASTASE INHIBITION

### Preclinical Studies

One of the major pathological findings in PAH is fragmentation of the pulmonary vascular internal elastic lamina, associated with SMC hyperplasia and neointima formation (40), occurring as early as four days after MCT injection in the rat (41, 42). Studies have shown that elastases (proteolytic enzymes that target elastin) can release growth factors from the extracellular matrix (43, 44). Treatment with oral serine elastase inhibitors reversed pulmonary vascular remodeling in MCT rats by inducing SMC apoptosis (45). Elafin is an endogenous elastase inhibitor with a strong anti-inflammatory profile; elafin-overexpressing mice were protected against hypoxia-induced pulmonary hypertension (46, 47). Subcutaneous recombinant human elafin improved hemodynamics, reduced occlusive pulmonary vascular lesions, and significantly increased the number of distal microvessels in the Sugen-hypoxia model (22). Complementary studies showed that elafin reduced the size of neointima in lesions through induction of SMC apoptosis in lung tissue from patients with PAH. Elafin also improved endothelial dysfunction by promoting cell survival and angiogenesis through novel signaling mechanisms involving BMPR2 and caveolin.

### Clinical Studies

The NIH has funded studies to advance the clinical development of elafin in PAH (HL108797). The proposed studies will assess the safety, tolerability, and efficacy of elafin and the effects on inflammatory markers.

## INFLAMMATION AND IMMUNITY

### Preclinical Studies

IL-6 is a pleiotropic cytokine that coordinates the inflammatory response in infection and tissue injury. Mice that overexpress IL-6 have increased RV systolic pressures, hypertrophy, and severe occlusive angioproliferative lesions in the small distal pulmonary vessels with infiltrating lymphocytes (48). IL-6-deficient mice are protected from hypoxia-induced pulmonary hypertension (49). More recently, pulmonary artery SMCs from experimental models were found to have ectopic upregulation of membrane-bound IL-6 receptor (IL-6R) (50); transgenic mice lacking the IL-6R on SMCs were protected against hypoxia-induced pulmonary hypertension and treatment with a IL-6R-specific antagonist reversed experimental pulmonary hypertension in two rat models (50). Elevated IL-6 modifies the expression of PAH in the dominant-negative *BMPR2* mutation mouse model, suggesting that IL-6 could act as a “second hit” in *BMPR2* mutation carriers leading to PAH (51).

Regulatory T cell (Treg) deficiency, dysregulated B cells, and pathogenic endothelial autoantibodies have been implicated in the pathogenesis of PAH. Athymic nude rats (which lack Tregs) exposed to Sugen-hypoxia showed pulmonary B cell accumulations and antiendothelial cell antibody deposition on the pulmonary vasculature, resulting in severe PAH (52). Immune reconstitution with healthy Tregs prevented B cell accumulation and the formation of antiendothelial cell antibodies as well as the development of PAH.

Dimethyl fumarate (DMF) is a NRF2 pathway activating agent that has been studied in the Sugen-hypoxia experimental model (53). DMF exerts potent anti-inflammatory effects in part by directly targeting the NF- $\kappa$ B signaling pathway through a covalent modification of p65 and is FDA-approved for use in multiple sclerosis. DMF reversed hemodynamic changes, reduced inflammation, and decreased oxidative stress in the Sugen-hypoxia model.

### Clinical Studies

IL-6 is increased in serum and lungs of patients with idiopathic PAH and PAH associated with autoimmune disorders and (along with IL-1) is associated with an increased risk of death (54, 55). Pulmonary arterial SMCs from patients with PAH have upregulated expression of IL-6R, and IL-6 appears to signal both via the membrane-bound (classic or *cis*) pathway and the IL-6 *trans*-signaling pathway (gp130) (50).

Tocilizumab is a humanized monoclonal antibody against the IL-6R that has been approved for the treatment of rheumatoid arthritis, Castleman’s disease, and juvenile arthritis. Following reports of PAH regression with tocilizumab, the Therapeutic Open Label Study of Tocilizumab in the Treatment of Pulmonary Arterial Hypertension (TRANSFORM-UK, NCT02676947) is a phase II open-label proof-of-concept study in which tocilizumab is administered once monthly for six months to PAH patients (excluding those with lupus erythematosus, rheumatoid arthritis, or mixed connective tissue disease) (56). Primary endpoints are safety and change in PVR. A pilot study of subcutaneous anakinra (which blocks IL-1) is also recruiting patients with PAH (NCT03057028).

While investigators are pursuing therapeutics for Tregs, rituximab is a chimeric monoclonal antibody against CD20 that targets B cells and has anecdotally been reported to be effective in CTD-related PAH, including systemic sclerosis–associated PAH. The ASCO1 study (NCT01086540) is a NIH-funded, double-blind, placebo-controlled phase II RCT evaluating the safety and efficacy of rituximab (two doses given two weeks apart) on disease progression in subjects with systemic sclerosis–associated PAH. The primary endpoint is the change in PVR at 24 weeks, and secondary endpoints include RV function measured by MRI.

Two current studies of drugs have targeted the NRF-2 pathway, including bardoxolone and DMF, in patients with systemic sclerosis–associated PAH (NCT02657356, NCT02981082).

## MITOCHONDRIAL DYSFUNCTION

### Preclinical Studies

The metabolic theory of PAH proposes that the multifaceted molecular abnormalities in PAH all lead to mitochondrial suppression in pulmonary vascular cells and extrapulmonary tissues (e.g., immune cells, RV cardiomyocytes, skeletal muscle), resulting in inhibition of glucose oxidation with secondary upregulation of glycolysis (57). We do not know whether these metabolic abnormalities cause PAH or are secondary to pulmonary vascular disease and subsequent RV failure. Mitochondrial dysfunction is characterized by inhibition of glucose oxidation due to inhibition of pyruvate dehydrogenase (PDH) after phosphorylation by pyruvate dehydrogenase kinase (PDK) as a result of hypoxia (HIF1 $\alpha$ ), inflammation, endoplasmic reticulum stress, and tyrosine kinase activation. The result of PDH inhibition is apoptosis resistance, proliferation, and inflammation, key features of PAH pathogenesis. The small molecule inhibitor dichloroacetate (DCA) activates PDH, reverses the glycolytic shift, and has prevented and reversed PAH in several animal models (58, 59).

### Clinical Studies

A recent four-month, open-label study of DCA (3–6 mg/kg b.i.d.) in 20 stable WHO functional class II–III idiopathic PAH patients suggested a reduction in mean PAP and PVR as well as an improvement in functional capacity in patients with SIRT3 and UCP2 variants that decrease PDH (60). However, larger RCTs of DCA are required to determine if this drug is effective and safe.

## OTHER METABOLIC PATHWAYS

Iron deficiency in nonanemic PAH patients is frequently observed (61, 62) and causes experimental pulmonary hypertension and pulmonary vascular remodeling in rats fed an iron-deficient diet (63). Extracellular iron is taken up by cells and transported to the mitochondria, where it is utilized for synthesis of cofactors in essential oxidation-reduction reactions. Several clinical trials investigating the role of iron supplementation in PAH and different modes of iron administration have been completed with promising results (NCT01447628, NCT02594917) (64, 65) or are still under way (NCT01447628, NCT02594917) (62).

Metformin improves endothelial function by increasing endothelial NO synthase phosphorylation and reduced pulmonary artery SMC proliferation (66); metformin also inhibits aromatase and estrogen production (67). Metformin protects against PAH in rats exposed to hypoxia or MCT purportedly through its antiproliferative properties (68), but antiestrogenic effects may also explain these findings (see the section titled Sex Hormones) (67). Metformin is currently being studied at Vanderbilt University for PAH (NCT01884051, NCT03617458).

The mechanistic target of rapamycin complex (mTORC) pathway promotes SMC proliferation (69). mTORC1 and mTORC2 pathways are upregulated in small remodeled pulmonary arteries and pulmonary arterial SMCs in PAH. Intervention studies showed that mTORC2 is required for adenosine triphosphate (ATP) generation and survival of idiopathic PAH pulmonary artery SMCs (70). mTORC2 downregulated the energy sensor adenosine monophosphate (AMP)-activated protein kinase, which leads to activation of mTORC1-S6 and increased proliferation, as well as a deficiency of the proapoptotic protein Bim. The mTOR kinase inhibitor PP242 suppressed mTORC2, induced SMC apoptosis in small pulmonary arteries, and reversed hypoxia-induced pulmonary vascular remodeling in rats. While mTORC2 inhibitors are being studied for certain cancers, there are currently no active studies in patients with PAH. A phase I clinical trial is using ABI-009 (a nanoparticle bound rapamycin targeted to the lungs) in PAH (NCT02587325).

Nitro-oleic acid (OA-NO<sub>2</sub>) prevents the development of endothelial dysfunction induced by either hypoxia or elevated levels of asymmetric dimethylarginine (71). These findings have recently led to the initiation of a phase II RCT to evaluate the safety, efficacy, and pharmacokinetics of CXA-10, a specific isomer of OA-NO<sub>2</sub>, in PAH patients with a focus on RVEF and hemodynamics as endpoints (NCT03449524).

## NERVOUS SYSTEM

Sympathetic nervous system activation, parasympathetic downregulation, and the renin-angiotensin-aldosterone system (RAAS) contribute to PAH in experimental models and patients with PAH (recently reviewed in 72, 73).

### Preclinical Studies

Release of catecholamines (locally or systemically) can cause pulmonary vasoconstriction by activating  $\alpha$ -1 receptors in the pulmonary vasculature (as does  $\beta$ -2 blockade), whereas inhibition of  $\alpha$ -receptors and parasympathetic activation lead to cholinergic-mediated relaxation. Under normal conditions,  $\beta$ -1 and  $\beta$ -2 receptors increase inotropy, chronotropy, and diastolic function through G protein receptors. Small studies in PAH have shown evidence of systemic sympathetic nervous activation. After initial compensation, the RV in PAH undergoes maladaptation and remodels with eventual downregulation of  $\alpha$ -1 and  $\beta$ -1 receptors and desensitization of RV cardiomyocytes (74, 75). Antagonism of adrenergic receptors prevents MCT-induced PH and decreases RV size (76, 77). Treatment of established RV failure in two different animal models with carvedilol reduced RV hypertrophy and fibrosis, decreased RV dilation, and increased RV capillarization. Carvedilol improved survival of the animals treated with MCT and affected profibrotic and



matrix remodeling pathways, cardiac hypertrophy, ceramide signaling, glucocorticoid receptor signaling, peroxisome proliferator-activated receptor, and NRF2. de Man et al. administered bisoprolol to the MCT model, showing an improvement in RV contractility, filling, and cardiac output and decreased inflammation and fibrosis while restoring  $\beta$ -adrenergic signaling (78). Nebivolol (which antagonizes  $\beta$ -1 receptors but serves as an agonist for  $\beta$ -2/3 signaling, producing vasodilation) improved RV hypertrophy and pulmonary vascular remodeling (79). Investigators have also used denervation procedures of the kidneys and lungs to reduce sympathetic stimulation with beneficial results in animal models (80, 81).

Patients with PAH have reduced heart rate recovery after exercise, which is linked to RVEF and decreased heart rate variability, reflecting decreased parasympathetic drive. RV and lung tissue from patients with PAH have decreased acetylcholinesterase levels; however, the RVs have increased nicotinic acetylcholine receptor expression (82). These findings may be compensatory but are insufficient to increase parasympathetic activity. Pyridostigmine improved parasympathetic activity (reflected by increased heart rate variability, improved spontaneous baroreflex sensitivity, and plasma acetylcholinesterase activity), delayed the time to right heart failure, reduced pulmonary vascular resistance, reduced RV wall thickness and end-diastolic diameter, and improved RV coupling in the Sugen-hypoxia rat model of PH. The pulmonary vasculature showed reduced intima and medial wall thickness, fewer occlusive lesions, and proliferation of microvascular endothelial cells.

### Clinical Studies

Several pharmacologic and nonpharmacologic interventions could target the autonomic system in patients with PAH. Observational studies of the use of  $\beta$ -blockers in cohorts of patients with various forms of PH did not show dramatic effects on disease severity or a significant increase in serious adverse events. Small pilot studies have administered low doses of  $\beta$ -blockers (carvedilol or bisoprolol) to patients with PAH; two of these studies included a placebo arm/period in a crossover study (83–85). In some of the studies, investigators saw decreased heart rates as well as decreases in cardiac index and 6MWD. Underlying RV function, including RV fractional area change, stroke volume, glycolytic rate, and  $\beta$ -adrenergic density, may have improved. The side effect profile included hypotension, fluid retention, and constitutional symptoms, as would be expected.

One placebo-controlled crossover trial of carvedilol is ongoing (NCT02507011). There remains clinical equipoise for larger phase II RCTs of  $\beta$ -blockade, likely using low doses, which minimize the acute hemodynamic effects while intervening in the downregulation of adrenergic receptors in the RV and lung vasculature. The early decreases in heart rate, cardiac output, and 6MWD, which are likely inevitable with  $\beta$ -blockade, would imply that longer-term clinical endpoints need to be the focus of such studies.

Researchers have proposed nonpharmacological alternatives to intervene on the adrenergic system, such as pulmonary artery denervation. Experimental models of ganglionic blocks or catheter ablation proximal to the main pulmonary artery bifurcation cardiopulmonary function have been effective. Nonrandomized studies of pulmonary artery denervation in patients with PAH have shown reductions in PAP and PVR as well as improvements in

exercise capacity and RV function (86, 87). Although this approach is intriguing, RCTs are needed to better understand its efficacy and safety (NCT02284737; NCT02525926). Longer-term studies are critical because neural regrowth could impact on the durability of the effect.

RCTs of pulmonary rehabilitation and training have shown benefit in terms of exercise capacity and even hemodynamic profile in PAH (88, 89). Although exercise affects a variety of signaling pathways, conditioning does increase parasympathetic tone, making this intervention attractive for future study.

## RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

### Preclinical Studies

The RAAS is closely related to autonomic function, and several studies show pathobiologic importance in PAH. Angiotensin II (Ang II) increases pulmonary perivascular fibrosis and RV collagen deposition. Angiotensin-converting-enzyme (ACE) inhibitors have been used for decades to treat other cardiac diseases, but small studies have not shown benefit in PAH. Expression of the Ang II receptor AT1 (which causes vasoconstriction, oxidative stress, inflammation, and proliferation) is increased in the pulmonary vasculature of patients with PAH (90). In this study, losartan reduced disease progression in the MCT model, restored RV-PA coupling, and improved RV diastolic function.

More recently, investigators have shown the importance of ACE2 in metabolizing Ang I and Ang II to Ang 1–7, Ang 1–9, and Ang 1–5, which activate the Mas receptor, leading to vasodilation, inhibition of proliferation, decreased inflammation, and activation of the parasympathetic system. Studies in preclinical models suggest that activation of ACE2 or administration of ACE2 and Ang 1–7 reduces RV pressure and hypertrophy, improves pulmonary vascular remodeling, and reduces levels of inflammatory biomarkers (91, 92). Diminazene aceturate is an ACE2 activator that also showed beneficial effects in experimental models (93).

Circulating aldosterone is elevated in experimental models of PAH and in some human studies (94). Administration of spironolactone to MCT and Sugen-hypoxia rats and hypoxic mouse models improved RV morphology and function and pulmonary vascular remodeling and reduced pulmonary arterial SMC proliferation (95, 96). Observational studies of the importance of aldosterone in patients with PAH have shown mixed results.

### Clinical Studies

One study administered a single dose of recombinant ACE2 intravenously to patients with PAH and showed an increase in cardiac output, reduction in PVR, and reduction in biomarkers of inflammation (97). A phase II dose-ranging study of recombinant ACE2 in PAH is ongoing (NCT03177603). Several clinical trials of spironolactone in patients with PAH have been completed or are active (NCT02253394, NCT01712620, NCT01468571).

## CELL-BASED THERAPY

### Preclinical Studies

Endothelial progenitor cells (EPCs) are circulating, bone marrow–derived cells that possess the ability to differentiate and mature into endothelial cells in specific settings. EPCs have the potential to hone in on areas of vascular injury and help repair and regenerate blood vessels to improve endothelial dysfunction and repair damage in pulmonary microvasculature. Preclinical studies in experimental models of PAH have shown that cell-based gene transfer of human eNOS prevents PAH in MCT rats (98). Furthermore, delayed eNOS EPC administration (i.e., 3 weeks after MCT) improved hemodynamics and regenerated the distal pulmonary microcirculation. While administration of non-eNOS EPCs also demonstrated some benefit, normalization of pulmonary pressures was seen only with eNOS-transduced cells, and this effect was associated with greater survival.

### Clinical Studies

A phase I dose-ranging safety trial, Pulmonary Hypertension and Cell Therapy (PHACeT; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00469027) Identifier: NCT00469027), was performed using autologous EPC transiently transfected by electroporation with plasmid DNA containing the full coding sequence of human eNOS (99). This study enrolled 7 patients (5 women) with a diagnosis of idiopathic PAH. Three received a total of 7 million cells (panel 1), 3 received 23 million cells (panel 2), and one patient received 50 million cells (panel 3) by central venous injection through the pacing port of a Swan-Ganz catheter over 3 days with continuous monitoring of pulmonary arterial pressures in an intensive care environment. No deterioration in pulmonary hemodynamics was seen during cell delivery. Investigators noted a trend toward improved total pulmonary resistance over the three-day delivery period, although no reduction in PAP or PVR was seen at 3 months. Significant improvements were noted in 6MWD, functional class, and quality-of-life measures, which are difficult to interpret in this unblinded study. One patient died during the study period.

The Study of Angiogenic Cell Therapy for Progressive Pulmonary Hypertension: Intervention With Repeat Dosing of eNOS-enhanced EPCs (SAPPHIRE; NCT03001414) includes two important design changes to increase the potential efficacy and improve the safety of eNOS gene-enhanced cell therapy for PAH. The first change is to repeat cell therapy on a monthly basis, increasing the cumulative cell dose that will be delivered over the 12-month study period. Thus, participants will receive 4 or 8 monthly injections of 20 million cells (depending on the study arm), for a maximum of 80 or 160 million cells, respectively. The second change is to use minicircle, rather than conventional bacterial plasmid, DNA for eNOS transfection. These advantages are expected to increase the magnitude and duration of the eNOS transgene expression, while also reducing the chance of host immune response.

## SUMMARY

Despite recent advances in treating patients with PAH with targeted therapies, PAH continues to cause significant morbidity and mortality. New discoveries derived from

experimental models and human studies are being tested as novel treatment approaches in patients. The continued translation of scientific discovery into therapies that act via new pathways will lead to the improved treatment of and an eventual cure for this disease.

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