

Advances in Therapeutic Interventions for Patients With Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a disease characterized by progressive remodeling of the distal pulmonary arteries, resulting in the loss of vascular cross-sectional area and elevated pulmonary vascular resistance (PVR).¹ Without intervention, PAH is usually progressive, leading to right heart failure and death. Since the first reported studies of calcium channel blockers >30 years ago,^{2,3} we have witnessed major advances in the therapeutic armamentarium available to treat this devastating condition. The development of drugs that specifically target pathways involved in disease pathogenesis has led to improvements in the quality of life and clinical outcomes in patients with PAH. At present, there are 10 drugs approved for PAH by regulatory authorities worldwide. This review will focus on recent advances in pharmacological therapy in adult PAH, including our current treatment approaches and potential future strategies.

Definition of Pulmonary Arterial Hypertension

PAH is an uncommon disease with an estimated prevalence of 15 to 50 per million population.^{4,5} Under the current clinical classification (Table 1),⁶ PAH consists of different etiologies leading to precapillary pulmonary hypertension (PH), which is defined by an end-expiratory mean pulmonary artery pressure (PAP) ≥ 25 mmHg, pulmonary artery wedge pressure ≤ 15 mmHg, and PVR >3 Wood units at rest.⁷ PAH is termed idiopathic (IPAH) when no causative factors are identified, but it can also be heritable, induced by drugs or toxins, or associated with conditions such as connective tissue disease, congenital heart disease, portal hypertension, and HIV infection. These conditions have been classified under PAH because they share common pathological changes in the small pulmonary arteries, and a similar therapeutic approach, as well.

Pathomechanisms and Treatment Targets

Endothelial Dysfunction – the Target of Current Therapies

Endothelial dysfunction leading to an imbalance of vasodilator and vasoconstrictor mediators is an important aspect of PAH pathogenesis. These pathways also exert, to a variable degree,

effects on vascular remodeling and coagulation homeostasis. The elucidation of molecules involved in the regulation of pulmonary vasomotion has led to the development of currently approved PAH therapies, which all target one of the following key pathways: (1) prostacyclin (PGI₂), (2) nitric oxide (NO), and (3) endothelin-1 (ET-1) pathways (Figure 1). PAH is characterized by sustained reduction in vasodilator mediators (such as PGI₂ and NO), whereas vasoconstrictors (such as ET-1) are upregulated.^{8–10}

Pulmonary Vascular Remodeling in PAH

From a pathological perspective, reduction in vascular luminal area results from a combination of arterial remodeling, inappropriate vasoconstriction, and in situ thrombosis.^{11,12} It has become apparent that arterial remodeling is perhaps the chief contributor to elevated PVR. All 3 layers of the arterial wall are involved, and typical arterial lesions in PAH consist of neointima formation and intimal fibrosis, medial hyperplasia of pulmonary artery smooth muscle cells (PASMC), and adventitial fibrosis accompanied by a variable degree of perivascular inflammation.¹³ Complex (or plexiform) lesions are the pathological hallmark of severe PAH and consist of exuberant expansion of endothelial cells, some of which display cancerlike features of monoclonal proliferation.¹⁴ It is increasingly recognized that patients with PAH may not exhibit pure arterial involvement, but also display venular remodeling: this observation is particularly frequent in systemic sclerosis-associated PAH.¹⁵

Vascular remodeling in PAH is a complex and multifactorial process, but recent advances have identified several key genetic, cellular, and molecular abnormalities.

Pulmonary vascular cells in remodeled arteries display a hyperproliferative and antiapoptotic phenotype, and factors such as excessive growth factor stimulation, alterations of bone morphogenetic proteins (BMPs)/transforming growth factor β signaling pathway, transcription factor abnormalities, and mitochondrial dysfunction all promote cell survival and proliferation.¹⁶ Perivascular inflammation is frequently encountered in pathological specimens, supporting its role in PAH pathogenesis and vascular remodeling.¹⁷ Abnormal ion

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Table 1. Pulmonary Arterial Hypertension in Current Clinical Classification (Nice, 2013)

Group 1 Pulmonary Arterial Hypertension (PAH)
1.1 Idiopathic
1.2 Heritable (<i>BMPR2</i> , <i>ALK-1</i> , <i>ENG</i> , <i>SMAD9</i> , <i>CAV1</i> , <i>KCNK3</i>)
1.3 Drugs or toxins induced
1.4 Associated with connective tissue disease, HIV infection, portal hypertension, congenital heart disease, schistosomiasis

ALK-1 indicates activin receptor-like kinase 1; BMPR2, bone morphogenetic protein receptor 2; CAV1, caveolin 1; ENG, endoglin; and HIV, human immunodeficiency virus.

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channel function and disrupted intracellular calcium homeostasis not only result in sustained vasoconstriction, but also induce PASM C proliferation.¹⁸ Finally, both qualitative and quantitative changes in endothelial progenitor cell population may contribute to disordered angiogenesis, although their exact role in PAH pathogenesis remains obscure.¹⁹

Genetic Susceptibility in PAH

Genetic background is increasingly recognized as an important predisposing factor in PAH and has provided significant insights into disease pathogenesis. The most common mutations involve genes encoding for the transforming growth factor β superfamily signaling pathways, of which mutations in bone morphogenetic protein receptor 2 (*BMPR-2*) account for $\approx 75\%$ of all heritable PAH cases. Other less common mutations of the transforming growth factor β superfamily include activin receptor-like kinase 1 (*ALK-1*) and endoglin (*ENG*), which are associated with PAH in the setting of hereditary hemorrhagic telangiectasia.^{20,21} Disease penetrance of *BMPR-2* mutation carriers is $\approx 20\%$, suggesting that other genetic/epigenetic abnormalities or appropriate environmental triggers contribute to PAH development. The importance of BMPR-II pathway in PAH pathogenesis is further underscored by the finding that BMPR-II expression is also reduced in the lungs of PAH patients who do not carry *BMPR-2* mutations.²² Although the precise mechanisms of how BMPR-II dysfunction leads to pulmonary vascular remodeling are still largely unknown, intact BMPR-II signaling appears important in antagonizing the growth-promoting response of PASM C to mitogenic stimuli such as platelet-derived growth factor (PDGF),^{23,24} and provides protection of pulmonary endothelial cells from apoptosis.²⁵

More recently, novel mutations have been identified such as those involving the potassium channel *KCNK3*.²⁶ *KCNK3* is expressed in PASM C and is important in the regulation of resting membrane potential and, hence, pulmonary vascular tone.^{27,28} It has been postulated that ion channels such as *KCNK3* may also play a role in cellular proliferation and vascular remodeling.²⁶

Basic Measures and Conventional Therapy

Exercise Rehabilitation

Exercise limitation is a debilitating symptom in PAH and is due to the inability of the right ventricle to augment cardiac

output through a restricted pulmonary circulation. Patients should be encouraged to stay active, but avoid over strenuous exertion beyond physical limits. Deconditioning and skeletal muscle dysfunction are common in PAH,²⁹ and recent randomized controlled trials (RCTs) support supervised rehabilitation in improving exercise capacity and quality of life.^{30–33} The optimal mode, intensity, and duration of exercise training are not clearly defined at present. The impact of cardiopulmonary rehabilitation on long-term outcomes is also unclear.

Oxygen Therapy

Hypoxemia in PAH can occur at rest, during exercise, and during sleep. Oxygen should be prescribed to maintain P_{aO_2} above 60 mmHg. Sleep-disordered breathing is common in precapillary PH and has been reported to occur in up to two-thirds of patients with PH and associated right heart failure.^{34,35} A recent small short-term randomized crossover trial of nocturnal oxygen therapy in PAH patients with sleep-related oxygen desaturation (but without daytime hypoxemia) demonstrated a small but significant treatment effect in 6-minute walk distance (6MWD) and right ventricular function.³⁶ Patients should avoid travel to >1500 m without supplemental oxygen and the need for in-flight oxygen needs to be assessed before air travel.

Anticoagulation

Previous small observational studies from the era of conventional therapy support a survival benefit of anticoagulation in IPAH,^{37–39} and this survival benefit has been recently suggested by a European Registry (COMPERA) study involving 1283 patients in the modern management era.⁴⁰ However, improvement in survival was restricted to IPAH but not other forms of associated PAH (such as systemic sclerosis). Despite the lack of RCT data, anticoagulation is recommended in PAH with vitamin K antagonist aiming for an international normalized ratio of 1.5 to 2.5, unless contraindicated. Because the evidence for anticoagulation in other forms of PAH is even weaker, careful risk-benefit assessment is warranted, especially in patients with systemic sclerosis who have a higher risk of bleeding.⁴¹ Anticoagulation is also warranted to minimize catheter-related thrombosis in those receiving continuous intravenous prostanoid therapy. In the absence of efficacy and safety data, it is premature to consider new oral anticoagulants (such as rivaroxaban) in PAH,⁴² and a recent RCT does not support the use of aspirin.⁴³

Calcium Channel Blockers

A small subset ($<10\%$) of patients with IPAH display favorable long-term response to high-dose calcium channel blockade. These patients can be identified by the presence of predefined hemodynamic response during acute vasoreactivity testing. Criteria for acute vasoreactivity are currently defined by a reduction in mean PAP ≥ 10 mmHg to achieve an absolute value of mean PAP <40 mmHg with an increased or unchanged cardiac output.⁴⁴ Vasoreactivity testing should only be performed with short-acting pulmonary vasodilators, and inhaled NO is currently the agent of choice based on established experience and evidence. Slow up titration but

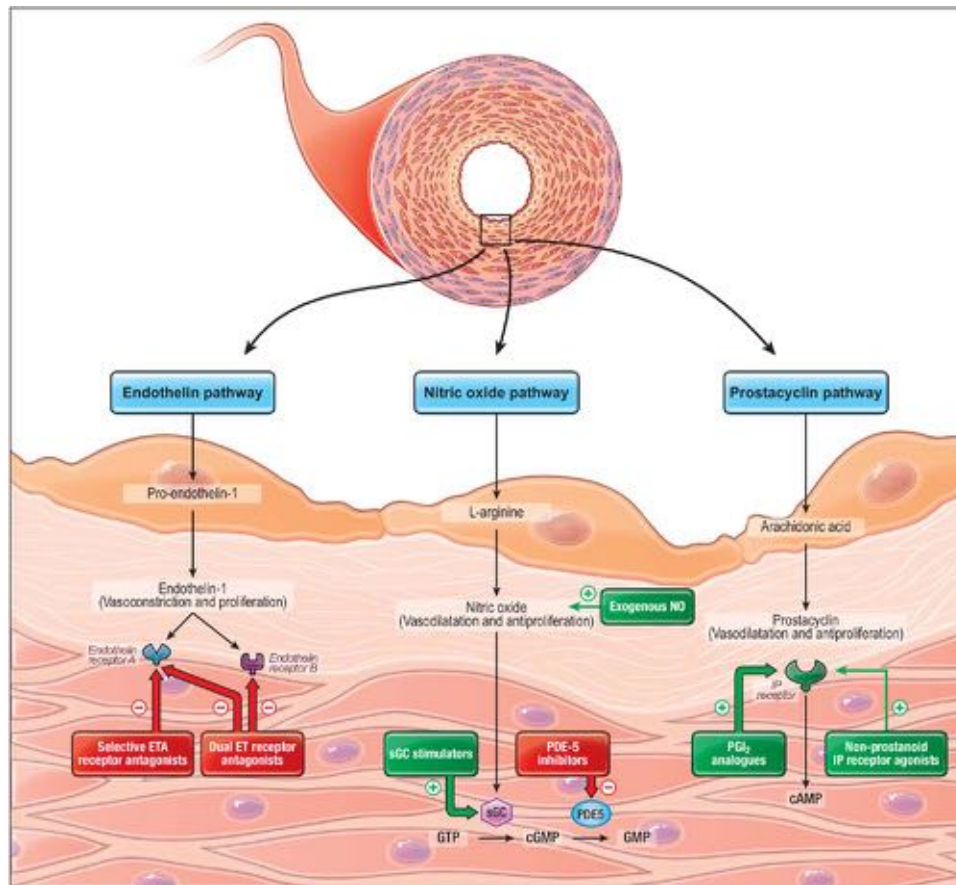


Figure 1. Established vasomotor pathways targeted by current and emerging therapies in PAH. The 3 major pathways (endothelin-1, nitric oxide, and prostacyclin) involved in the regulation of pulmonary vasomotor tone are shown. These pathways represent the targets of all currently approved PAH therapies. Endothelial dysfunction results in decreased production of endogenous vasodilatory mediators (nitric oxide and prostacyclin) and the upregulation of endothelin-1, which promotes vasoconstriction and smooth muscle cell proliferation. The endothelin-1 pathway can be blocked by either selective or nonselective endothelin-1 receptor antagonists; the nitric oxide pathway can be manipulated by direct administration of exogenous nitric oxide, inhibition of phosphodiesterase type-5, or stimulation of soluble guanylate cyclase; and the prostacyclin pathway can be enhanced by the administration of prostanoid analogues or nonprostanoid IP receptor agonists. ET indicates endothelin; ETA, endothelin type A; IP, prostaglandin I₂; NO, nitric oxide; PAH, pulmonary arterial hypertension; PDE-5, phosphodiesterase type 5; and sGC, soluble guanylate cyclase.

high-maintenance dosages of calcium channel blockers are required (typically diltiazem, 240–720 mg; nifedipine, 120–240 mg). Patients who do not achieve sustained hemodynamic response and New York Heart Association (NYHA) functional class (FC) I to II status require other PAH therapy.

Other Basic Measures

Judicious use of diuretics remains indispensable to provide symptomatic relief via reduction of right ventricular preload and improvement in left ventricular filling. Supraventricular arrhythmias are poorly tolerated in severe PAH and their management is often difficult. Early electric cardioversion should be considered, and antiarrhythmic drugs such as amiodarone can be used to maintain sinus rhythm.⁴⁵ Digoxin is useful for rate control, but drugs with negative inotropic properties should be avoided. Referral to an electrophysiologist should be considered, particularly in the setting of atrial flutter where ablation has a high success rate of preventing recurrence.

The mortality rate associated with pregnancy in PAH is high and has been reported at 17% to 56%.⁴⁶ Therefore, pregnancy should generally be avoided and early termination

recommended for those who become pregnant. In selected cases, pregnancy might be feasible for those who achieve near-normalization of hemodynamics during therapy.⁴⁷ Patients choosing to continue pregnancy should be managed within a multidisciplinary team in an expert PH center. ET-1 receptor antagonists and warfarin cannot be used during pregnancy owing to teratogenicity. Progesterone-based hormone contraception is preferred over estrogens because of the theoretical increased risk of thromboembolism. The ET-1 receptor antagonist bosentan induces cytochrome p450 activity and reduces the efficacy of hormonal contraceptives, and additional modes of contraception are required.⁴⁶ Genetic counseling and psychosocial support are also important elements of supportive care and should be offered as appropriate. Figure 2 summarizes the components of basic measures in PAH.

Approved PAH Therapies

Prostacyclin Pathway

Endogenous PGI₂ is produced mainly by endothelial cells from arachidonic acid via prostacyclin synthase and preferentially binds to prostaglandin I (IP) receptors with downstream effect

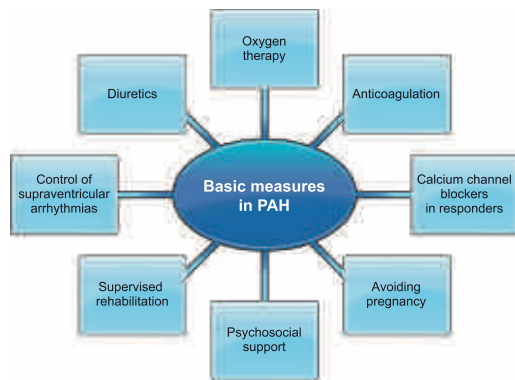


Figure 2. Basic measures and conventional therapies in PAH. The components of basic measures in the management of PAH are shown. Calcium channel blockers should only be used in patients who fulfill criteria for acute vasoreactivity (see text). PAH indicates pulmonary arterial hypertension.

on cAMP levels. PGI₂ has vasodilator, antithrombotic, anti-inflammatory, and antiproliferative effects on the vessel wall.⁴⁸

Epoprostenol

Intravenous (IV) epoprostenol is a prostacyclin analogue and was the first targeted therapy approved for the treatment of PAH. It remains the only drug with demonstration of survival benefit in a RCT. In this pivotal 12-week trial of 81 patients with severe IPAH in NYHA FC III to IV, epoprostenol decreased PVR, improved 6MWD, and, importantly, no patient in the epoprostenol group died in comparison with 8 patients in the conventional treatment group ($P=0.003$).⁴⁹ As such, IV epoprostenol is still considered the gold standard therapy in severe PAH and has reduced the need for lung transplantation in patients with advanced disease. However, administration of IV epoprostenol is complex, and it must be given as a continuous infusion via an indwelling catheter with its associated potential complications. Patients and caregivers require counseling on catheter care and the prevention of catheter-related infections.⁵⁰ Furthermore, abrupt interruption of therapy may result in potentially life-threatening rebound PH. A new formulation of IV epoprostenol that remains stable in solution for 48 hours at room temperature is now available, reducing the frequency of infusion pump change and improving patient convenience.⁵¹

Iloprost

Iloprost can be delivered via both inhaled and IV routes, although the inhaled route has only been formally tested in RCTs. Inhaled iloprost has a short half-life ($t_{1/2} \approx 25$ minutes) and must therefore be given at least 6 times/d to achieve clinical efficacy. In the multicenter AIR study⁵² that included patients with IPAH, connective tissue disorder-PAH, and inoperable chronic thromboembolic PH, treatment response (defined as FC improvement together with at least 10% increase in 6MWD) was displayed in 17% of iloprost-treated patients versus 5% of placebo at the end of 12 weeks.

Treprostinil

Treprostinil has greater chemical stability and longer half-life than epoprostenol. Its pharmacokinetic characteristics

enable administration via the IV, subcutaneous (SC), inhaled, and oral routes. Continuous SC treprostinil avoids the practical difficulties and complications associated with indwelling central venous catheters. However, some patients may experience severe infusion site pain requiring discontinuation. The clinical efficacy of SC treprostinil was examined in a large RCT involving 470 patients with IPAH, connective tissue disease-PAH, and congenital heart disease-PAH.⁵³ SC treprostinil improved exercise capacity as measured by 6MWD and treatment response appeared dose dependent.

Patients can be safely transitioned from IV epoprostenol therapy to both SC or IV treprostinil.^{54,55} IV treprostinil has been associated with an increased risk of catheter-related blood stream infection, possibly attributed to the neutral pH of its diluent solution.⁵⁶ Because of the risks associated with chronic indwelling central venous catheters, IV treprostinil should be reserved for patients who are intolerant of the SC route, or in whom these risks are considered warranted. IV treprostinil requires greater equivalent dosages than IV epoprostenol and, hence, higher related expense.

Oral treprostinil (an extended release formulation) has been assessed in 1 RCT in treatment-naïve patients (Oral Treprostinil as Monotherapy for the Treatment of Pulmonary Arterial Hypertension [FREEDOM-M]) and 2 combination therapy RCTs (Oral Treprostinil in Combination with an ERA and/or PDE-5I for Treatment of PAH [FREEDOM-C and FREEDOM-C2]). In the 12-week FREEDOM-M study,⁵⁷ oral treprostinil significantly improved 6MWD in comparison with placebo (+23 m in comparison with baseline, $P=0.0125$) but had no effect on clinical worsening. The average dose achieved was 3.4 mg orally, corresponding to the equivalence of low-dose parenteral therapy at ≈ 10 to 30 ng·kg⁻¹·min⁻¹. However, both combination therapy trials (FREEDOM-C and FREEDOM-C2), where background therapy with nonprostanoids was allowed, failed to meet their primary end point in 6MWD.^{58,59}

Beraprost

Beraprost is an orally active prostanoid and is only licensed for use in Japan and South Korea. Despite the initial demonstration of improvement in 6MWD and symptoms in a short-term trial,⁶⁰ the beneficial effects of beraprost were not sustained in a longer-term 12-month study.⁶¹

Endothelin Pathway

ET-1, released predominantly from endothelial cells, is one of the most potent vasoconstrictors known in biology and exerts its effects on 2 distinct receptor subtypes, ETA and ETB receptors. ETA receptor is localized to PASM, whereas ETB receptor is found predominantly on the endothelium but is also present on PASM. Smooth muscle constriction and proliferation are mediated by both ETA and ETB receptor isoforms,⁶² but ETB receptor is involved in the local clearance of ET-1 and can induce vasodilation via the release of NO and PGI₂ from endothelial cells.⁶³ In addition to promoting pulmonary artery endothelial cells and PASM proliferation, ET-1 also induces fibroblast activation, contraction, and synthesis of the extracellular matrix.⁶⁴

Bosentan

Bosentan is a nonselective dual ET-1 receptor antagonist and was the first oral agent approved for the treatment of PAH. The clinical efficacy of bosentan was shown in the Bosentan Randomized Trial of Endothelin Antagonist Therapy (BREATHE-1) RCT that enrolled 213 patients in NYHA FC III to IV.⁶⁵ Bosentan was associated with a gain of 44 m in 6MWD and improvement in FC status at 16 weeks. Bosentan has also been studied specifically in congenital heart disease-PAH in the BREATHE-5 study and improved both exercise capacity and hemodynamics.⁶⁶ Liver transaminase elevation is a common side effect. In postmarketing surveillance studies, the reported annual rate of liver transaminase elevation above 3 times the upper limit of normal was 10.1%, and the discontinuation of therapy was required in 3.2% of patients.⁶⁷ Thus, monthly monitoring of liver function tests is mandatory during bosentan therapy, but hepatic injury is fully reversible on drug cessation.

Ambrisentan

The selective ETA receptor antagonist ambrisentan was evaluated in the Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy Study 1 and 2 (ARIES-1 and ARIES-2) RCTs with demonstration of improvements in 6MWD and NYHA FC.⁶⁸ Risk of liver injury related to ambrisentan is low,⁶⁹ and there is also a reduced potential for drug-drug interaction in comparison with bosentan. Peripheral edema is a common side effect of ambrisentan therapy. Despite the theoretical mechanistic advantage of selective ETA receptor blockade (because of the role played by ETB receptor in local ET-1 clearance), clinical efficacy appears broadly similar between bosentan and ambrisentan.

Macitentan

Macitentan is a dual ET-1 receptor antagonist with modification of the structure of bosentan to achieve high lipophilicity, increased receptor affinity, and prolonged receptor binding.^{70,71} As a result, macitentan has enhanced tissue penetration with sustained antagonism against ET-1 receptors.

Macitentan was evaluated in the recent phase III Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome (SERAPHIN) trial,⁷² a morbidity and mortality, event-driven, placebo-controlled trial that represented a significant departure from the traditional short-term 6MWD end point characteristic of earlier PAH trials. The primary end point was time from the initiation of treatment to the first clinical event, which was the composite of all-cause mortality, transplantation, atrial septostomy, initiation of parenteral prostanoids, or clinical worsening of PAH defined by the presence of all 3 criteria: (1) $\geq 15\%$ decline in 6MWD, (2) worsening symptoms of right heart failure or increase in NYHA FC, and (3) the need for new PAH therapy excluding oral diuretics. Clinical worsening of PAH was adjudicated by an independent review committee. In this study, 742 PAH patients were randomly assigned to macitentan at 3 mg daily, 10 mg daily, or placebo with an average exposure duration of ≈ 100 weeks. Almost all patients were in NYHA FC II to III (52% FC II and 46% FC III), and 64% of patients were on background therapy.

Kaplan-Meier estimates for the first PAH-related clinical event showed a significant treatment effect favoring macitentan at a daily dose of 3 mg versus placebo (hazard ratio, 0.70; $P=0.01$) and macitentan at a daily dose of 10 mg versus placebo (hazard ratio, 0.55; $P<0.001$). This positive treatment effect was preserved irrespective of the presence of background therapy. The primary end point was driven mainly by worsening of PAH, and all-cause mortality as the first event was not significantly different between macitentan and placebo (on all-cause mortality the hazard ratio was 0.64 up to the end of treatment and 0.77 up to the end of the study for macitentan 10 mg versus placebo). Liver function abnormalities and peripheral edema were similar across all treatment groups, but the macitentan-treated group was associated with a higher incidence of anemia.

The SERAPHIN trial is pivotal, because it was the first study to adopt robust and clinically relevant end points that reflect PAH disease progression. Furthermore, the long-term nature of the study will provide invaluable information regarding the natural history and survival of PAH in the current management era without the inherent biases associated with data from open-label studies or registries.⁷³

Sitaxentan

Sitaxentan, a selective ETA receptor antagonist, was withdrawn from the market in 2010 because of an increasing number of deaths attributed to acute liver toxicity.⁷⁴

Nitric Oxide-cGMP Pathway

A key feature of endothelial dysfunction is reduced NO production and bioavailability in endothelial cells. NO is produced from L-arginine via the enzymatic action of nitric oxide synthase (NOS) and activates soluble guanylate cyclase (sGC), which then catalyzes the formation of the second messenger cyclic guanine monophosphate (cGMP), resulting in smooth muscle relaxation. NO is also an inhibitor of smooth muscle proliferation and platelet activation. This pathway can be manipulated via direct administration of NO, inhibition of phosphodiesterase-5 (PDE-5) (the enzyme responsible for cGMP degradation) or stimulation of sGC.

Sildenafil

The intracellular degradation of cGMP is regulated by PDEs, and PDE-5 plays a key role in the regulation of smooth muscle tone in the pulmonary vascular bed and the corpus cavernosum.⁷⁵ Sildenafil and tadalafil are reversible competitive inhibitors of the catalytic domain of PDE-5 involved in the hydrolysis of cGMP.

Sildenafil was the first PDE-5 inhibitor approved for the treatment of PAH and its efficacy was demonstrated in the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER-1) RCT that enrolled 278 patients in NYHA FC II to III to receive either placebo or sildenafil at 20 mg, 40 mg, and 80 mg 3 times daily for 12 weeks.⁷⁶ All 3 doses were associated with similar treatment effects in 6MWD, and sildenafil is licensed for PAH at a dose of 20 mg 3 times a day. The open-label extension study (SUPER-2) demonstrated that the majority (60%) of patients improved or maintained their functional status at 3 years of treatment at the unapproved dose of 80 mg 3 times a day.⁷⁷

Tadalafil

Tadalafil is structurally distinct in comparison with sildenafil, which accounts for its different pharmacokinetic properties and longer half-life. Tadalafil was assessed in the Tadalafil in the Treatment of Pulmonary Arterial Hypertension (PHIRST-1) RCT where 405 patients (NYHA FC II–III) were randomly assigned to 2.5 mg, 10 mg, 20 mg, or 40 mg daily of tadalafil for 16 weeks.⁷⁸ Tadalafil increased 6MWD in a dose-dependent manner but only the 40-mg dose met the pre-specified level of statistical significance with an overall treatment effect of 33 m. Background therapy with bosentan was present in 53% of study subjects, but statistically significant improvement in 6MWD was not achieved in the combination therapy subgroup.

Riociguat

sGC is the key catalytic enzyme involved in generation of the signaling molecule cGMP. Compounds acting on sGC are termed stimulators or activators depending on whether they target sGC in its NO-sensitive reduced state or NO-insensitive oxidized state, respectively.⁷⁹ Riociguat is a sGC stimulator and stabilizes sGC in its active configuration at low levels of NO availability but is also capable of cGMP production even in the absence of NO.⁸⁰ This is important because PAH patients display deficiency in endothelial NOS and, hence, NO production. The combined effect of riociguat and NO is more pronounced than either compound alone.⁸¹

Riociguat has been studied in the phase III Pulmonary Arterial Hypertension Soluble Guanylate Cyclase–Stimulator Trial 1 (PATENT-1) RCT.⁸² This was a 12-week double-blind, placebo-controlled trial that enrolled 443 symptomatic PAH patients assigned in a 2:4:1 ratio to placebo, riociguat titrated to maximum dose of 2.5 mg 3 times/d, and riociguat titrated to 1.5 mg 3 times/d, respectively. The 1.5-mg group was exploratory and was not included in the primary efficacy analysis of the trial. The traditional 6MWD was used as the primary end point, and secondary end points included PVR, N-terminal pro-brain natriuretic peptide, NYHA FC, and time to clinical worsening. Background therapy with either an ET-1 receptor antagonist or prostanoid was allowed, and nearly all patients were in NYHA FC II to III. At 12 weeks, riociguat increased 6MWD in comparison with placebo with a mean treatment effect of +36 m (95% confidence interval, 20–52 m). The primary end point in 6MWD was achieved in both treatment-naïve and combination-therapy groups. The secondary end point of pulmonary hemodynamics, NYHA FC, and time to clinical worsening also favored the riociguat-treated group. Riociguat was well tolerated, and the most serious adverse effects were hypotension, syncope, and bleeding (including hemoptysis). Similar efficacy and safety of riociguat were also demonstrated in the companion trial on inoperable chronic thromboembolic PH (Chronic Thromboembolic Pulmonary Hypertension Soluble Guanylate Cyclase–Stimulator Trial 1 [CHEST-1]).⁸³

Potential therapeutic synergy between PDE-5 inhibitors and sGC stimulators has been proposed based on the observation that cGMP can activate PDE-5 as a normal regulatory feedback loop.⁸⁴ The combined use of sildenafil and riociguat was explored in the PATENT-PLUS study, but this was associated

with higher rates of discontinuation due to systemic hypotension without evidence of clinical benefit in comparison with sildenafil alone.⁸⁵ With riociguat, the concurrent use of PDE-5 inhibitors is not recommended.

New Agents Targeting Established Vasodilatory Pathways

Nonprostanoid IP Receptor Agonists

Selexipag is an orally available nonprostanoid diphenylpyrazine derivative and acts on the human prostaglandin I₂ (IP) receptor. Unlike prostacyclin analogues, it has high specificity for the IP receptor and, hence, reduced theoretical side effects related to activation of other prostanoid receptors that mediate unwanted side effects. The active metabolite of selexipag has a prolonged half-life in comparison with prostanoid analogues and permits twice daily dosing.

The efficacy of selexipag has been evaluated in a multicenter phase II RCT involving 43 patients with PAH in NYHA FC II to III on background therapy with either an ET-1 receptor antagonist or sildenafil or both.⁸⁶ The primary end point was hemodynamic as assessed by PVR at 17 weeks. Selexipag led to a significant treatment effect of 30.3% reduction in PVR in comparison with placebo ($P=0.0045$). 6MWD was not significantly different between the groups but favored selexipag with an improvement of 25 m from baseline. Treatment appeared well tolerated, and 63.6% of selexipag-treated patients achieved a final optimal dose of 600 mg twice daily or higher. These encouraging findings prompted the recently completed Prostacyclin (PGI₂) Receptor Agonist In Pulmonary Arterial Hypertension (GRIPHON) study, a phase III double-blind, placebo-controlled, event-driven, morbidity and mortality trial that enrolled 1156 PAH patients. Selexipag decreased the risk of a morbidity/mortality event versus placebo by 43% ($P<0.0001$) and patients were treated for up to 4.3 years.⁸⁷ The full results of this study are expected to be available by the end of 2014.

Nitrite-NO Pathway

Although inhaled NO is already in clinical use for acute vasodilator testing and is licensed for the treatment of persistent PH in the newborn, domiciliary use in PAH has been hampered by the need for complex delivery systems and the availability of effective oral therapies.

Apart from the classical L-arginine-NOS-NO pathway, NO can also be generated via an alternative NOS-independent pathway involving the anion nitrite (NO₂⁻).⁸⁸ NO₂⁻ can be converted to NO in the lung by multiple enzymes with NO₂⁻ reductase activity such as xanthine oxidoreductase, aldehyde reductase, and deoxyhemoglobin.⁸⁹ In an ovine model of hypoxia-induced pulmonary vasoconstriction, inhaled NO₂⁻ resulted in potent pulmonary vasodilatation without an effect on systemic blood pressure.⁹⁰ Treatment with inhaled nitrite in monocrotaline- and hypoxia-induced rodent models can also prevent and reverse PH. NO₂⁻ is chemically more stable than NO and has a half-life of 30 minutes, which has implications for potential clinical translation because intermittent dosing is possible. The acute hemodynamic effect of inhaled nitrite is currently being investigated in human PAH (NCT01431313).

Inhibition of Cyclic Nucleotides Metabolism

The cyclic nucleotides (cGMP and cAMP) regulate vasomotor tone and vascular smooth muscle proliferation. In addition to degradation via PDEs, intracellular cyclic nucleotides levels are also regulated by an active efflux transport system. Multidrug resistance-associated protein 4 (MRP4), a member of the ATP-binding cassette transporter family, mediates the

cellular efflux of cyclic nucleotides, and the transport of other endogenous metabolites and drugs, as well.⁹¹

Recently, MRP4 was found to be overexpressed in the pulmonary arteries from PAH patients and also in mice exposed to hypoxia. Administration of a MRP4 inhibitor (MK571) reversed hypoxia-induced PH in mice. In contrast, MRP4 knockout mice are protected from hypoxia-induced

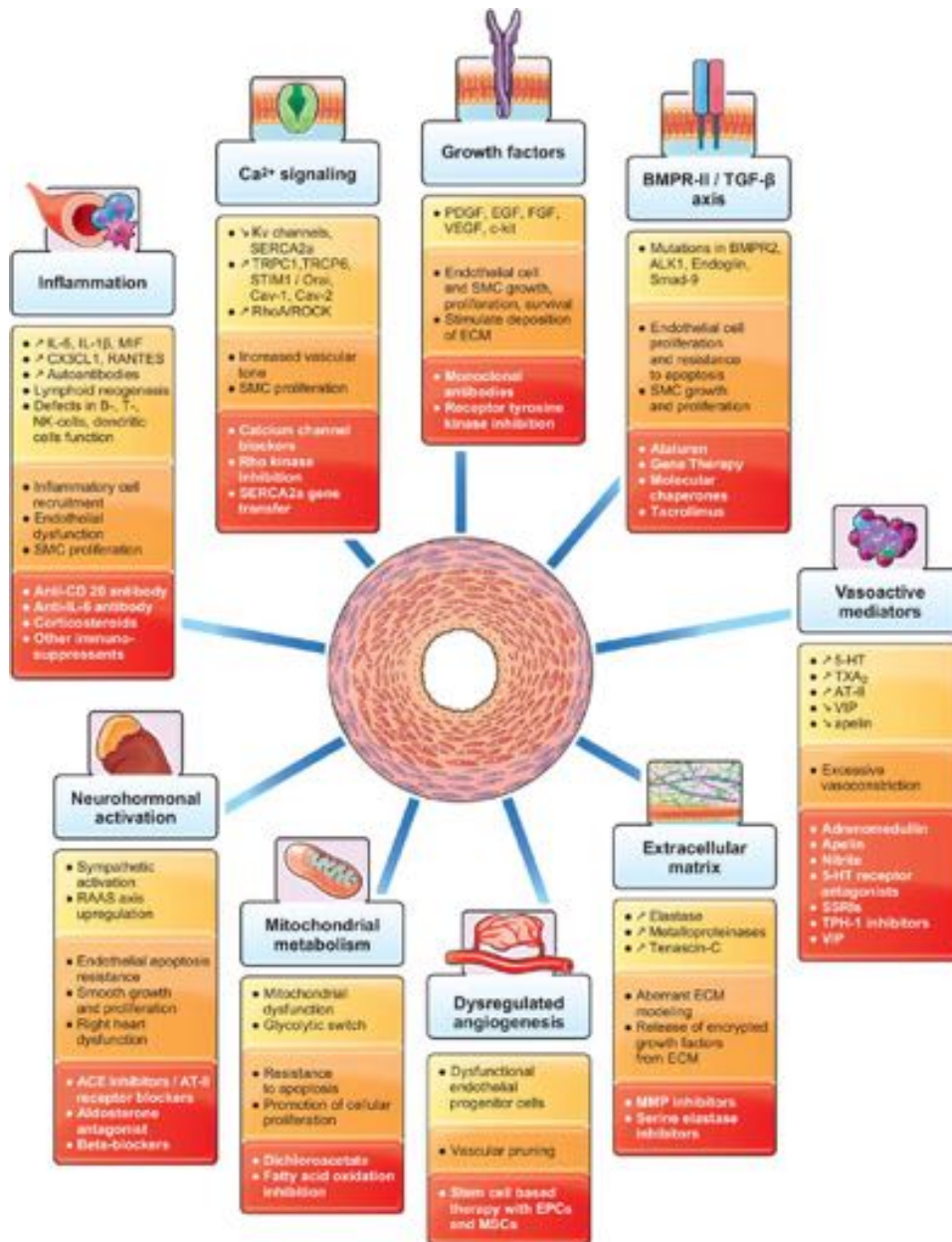


Figure 3. Novel therapeutic targets in PAH. An improved understanding of the molecular, cellular, and genetic mechanisms leading to PAH have provided translational opportunities for the development and testing of novel therapeutic agents. Various emerging pathways amenable to therapeutic manipulation are summarized. An evolving concept is the adoption of strategies with antiproliferative, reverse-remodeling, and regenerative effects on the pulmonary vasculature, as current PAH therapies have predominant vasodilatory properties and do not provide a cure for the condition. ACE indicates angiotensin-converting enzyme; ALK-1, activin receptor-like kinase 1; AT-II, angiotensin II; BMPR2, bone morphogenetic protein receptor type 2; CAV, caveolin; ECM, extracellular matrix; EGF, epidermal growth factor; EPCs, endothelial progenitor cells; FGF, fibroblast growth factor; 5-HT, serotonin; MIF, macrophage migration-inhibitory factor; MMP, matrix metalloproteinase; MSCs, mesenchymal stem cells; PAH, pulmonary arterial hypertension; PDGF, platelet derived growth factor; RAAS, renin-angiotensin-aldosterone system; SERCA2a, sarcoplasmic reticulum Ca²⁺-ATPase2a; SMC, smooth muscle cell; SSRIs, selective serotonin reuptake inhibitors; TPH-1, tryptophan hydroxylase-1; TRPC, transient receptor potential channels; TXA₂, thromboxane A₂; VEGF, vascular endothelial growth factor; and VIP, vasoactive intestinal peptide.

pulmonary vascular remodeling and also display reduced inflammatory cell recruitment to pulmonary arteries.⁹² Taken together, inhibition of MRP4 might represent an interesting target for human PAH.

Novel Pathways in PAH

Recent advances in our understanding of the molecular and cellular basis that govern pulmonary vasomotor tone and vascular remodeling have provided translational opportunities for novel therapeutic targets in PAH (Figure 3).

Tyrosine Kinase Inhibition

The pro-proliferative and antiapoptotic phenotype characteristic of PAH vasculopathy has led to interest in the role of growth factors in this disease. PDGF appears to be a key player in this regard. PDGF-A and PDGF-B are upregulated in animal models of PAH⁹³ and in remodeled arteries of human PAH.⁹⁴ Furthermore, both pulmonary endothelial cells and PASMCs from PAH patients display enhanced proliferation and survival in response to PDGF stimulation in comparison with controls.⁹⁵ Other growth factors implicated in the pathogenesis of PAH include fibroblast growth factors, epidermal growth factors, and vascular endothelial growth factors.⁹⁶ These growth factors all act via transmembrane receptor tyrosine kinases with resultant downstream activation of complex signaling networks that mediate a diverse range of cellular functions, including differentiation, migration, growth, and apoptosis.^{96,97}

The importance of growth factors in pulmonary vascular remodeling suggests that tyrosine kinase inhibition is a potentially rewarding antiproliferative strategy in PAH. Tyrosine kinase inhibitors (TKIs) have revolutionized the management of numerous oncological conditions, and the enhanced proliferative but suppressed apoptotic phenotype of pulmonary vascular lesions displays numerous parallels with cancer.⁹⁶

Imatinib

Imatinib is a nonspecific TKI with activity against the bcr-abl fusion protein (responsible for Philadelphia chromosome positive chronic myeloid leukemia), PDGFR, and c-kit. Prompted by the encouraging results of a small phase II study where imatinib improved pulmonary hemodynamics in a subgroup of patients with severe disease (defined as PVR >1000 dyn·s·cm⁻⁵),⁹⁸ the phase III Imatinib in Pulmonary Arterial Hypertension Randomized Efficacy Study (IMPRES) RCT was subsequently conducted.⁹⁹ In this study, 103 PAH patients were randomly assigned to imatinib, and 99 patients were randomly assigned to placebo over 24 weeks, and inclusion criteria specified that patients were already heavily pretreated with at least 2 PAH drugs (ET-1 receptor antagonists, PDE-5 inhibitors, or PGI₂ analogues) for ≥3 months in conjunction with a PVR ≥800 dyne·s·cm⁻⁵ at enrollment. At 24 weeks, a treatment effect of +32 m (P=0.002) in 6MWD was found favoring the imatinib group. However, interpretation of this result is difficult because of the differential dropout rate in the 2 arms (33% in imatinib-treated group versus 18% in the placebo group). Furthermore, no improvements in NYHA FC or time to clinical worsening were observed. Importantly, treatment with imatinib was poorly tolerated and serious adverse

events were common, including the occurrence of subdural hematomas. Two cases of subdural hematomas were reported in the 24-week randomized period and a further 6 additional cases occurred in the open-label phase of the study, representing 4.2% of imatinib-treated patients. However, the IMPRES study suggested that imatinib, similar to previous reports, was able to improve cardiac output with a modest reduction of mean PAP in a patient population with advanced disease despite therapy with at least 2 targeted PAH drugs. The unfavorable risk-benefit ratio of imatinib has resulted in its cessation from further therapeutic development in PAH, and its off-label use in PAH is not encouraged.

An additional concern with TKIs is cardiotoxicity. Both experimental data and human reports suggest that many TKIs can induce cardiovascular side effects, which include left ventricular dysfunction, conduction abnormalities, acute coronary syndromes, myocardial injury, arterial thromboses, and systemic hypertension.^{100–102} Although the mechanisms remain poorly understood, it is likely that pathways that promote pathological survival and proliferation of cancer cells may also regulate homeostasis of normal cells, including cardiomyocytes.¹⁰³ Targeting these pathways may inherently lead to collateral cardiotoxicity, because of the inhibition of receptor tyrosine kinases required for normal cardiomyocyte survival.

Another paradoxical feature of TKIs is the recent finding that dasatinib, used in the treatment of refractory chronic myeloid leukemia with broad activity against a wide array of targets (bcr-abl, PDGFR, c-kit, and Src family kinases) can actually induce PAH. The French registry reported 9 cases of dasatinib-induced PAH, and clinical improvements were observed following withdrawal of dasatinib.¹⁰⁴ Of note, all patients had received imatinib before dasatinib, and 6 patients were subsequently switched to nilotinib without the occurrence of PAH. It is possible that inhibition of Src kinase may influence pulmonary vascular tone, and it has recently been demonstrated that Src is involved in the control of the potassium channel KNCK3 and resting membrane potential in PASMC.¹⁰⁵

Targeting growth factors remains an attractive and biologically rational therapeutic strategy in PAH, but a better understanding of the interaction between the pathobiology of PAH and growth factors will be required to facilitate the development of new drugs that specifically block PAH pathways without unwanted side effects.¹⁰⁶

Serotonin System

The appetite-suppressive drugs (aminorex, dexfenfluramine, benfluorex) are indirect serotonergic agonists and serotonin transporter substrates,^{107,108} and the outbreak of PAH induced by these drugs implicated involvement of the serotonin system in PAH pathogenesis. Serotonin causes pulmonary artery vasoconstriction and induces PASMC proliferation. In IPAH, platelet storage of serotonin is depleted, peripheral blood serotonin levels are elevated,¹⁰⁹ pulmonary endothelial cells secrete more serotonin, and PASMCs demonstrate enhanced serotonin transporter expression and proliferate more in response to serotonin stimulation.¹¹⁰ A phase II study of tergruride, a 5HT-2 receptor antagonist, has been completed.¹¹¹

Overall, terguride was not associated with any improvements in either hemodynamics or exercise capacity.

De novo synthesis of serotonin from tryptophan is catalyzed by tryptophan hydroxylase-1 (TPH-1). Overexpression of the TPH-1 gene is found in remodeled arteries of IPAH patients¹¹² and hypoxia-induced PH is attenuated in TPH^{-/-} mice.¹¹³ Inhibition of TPH-1 is a potential target against the serotonin system in PAH.

Vasoactive Intestinal Peptide

Vasoactive intestinal peptide is a 28 amino acid originally discovered in the gut that mediates smooth muscle relaxation via activation of adenylate cyclase and generation of cAMP. Despite initial reports of hemodynamic and functional improvements in small uncontrolled studies,¹¹⁴ a phase II RCT of inhaled vasoactive intestinal peptide in 56 PAH patients was unable to demonstrate any beneficial effects both in hemodynamics or exercise capacity.¹¹⁵ No further trials of vasoactive intestinal peptide in PAH are currently ongoing.

RhoA/Rho-kinase Signaling Pathway

Rho-kinase (ROCK) is the downstream effector of the small GTPase RhoA and mediates a diverse range of cellular function including cell migration, smooth muscle contraction, cytokinesis, and gene expression.¹¹⁶ In the pulmonary arteries of PAH, the RhoA/ROCK signaling pathway is activated and ROCK can lead to smooth muscle hypercontractility by suppressing myosin phosphatase activity which controls Ca²⁺ sensitivity of myofilaments.¹¹⁷ In addition, the effect of serotonin on PASMC proliferation is, in part, mediated through increased RhoA/ROCK activity.¹¹⁸

Fasudil is a potent specific ROCK inhibitor and acute IV administration leads to a 17% reduction in PVR in a small observation study involving 9 patients with severe PAH.¹¹⁹ A long-acting oral formulation of fasudil was recently tested in a small pilot RCT in Japan involving 23 PAH patients over 12 weeks.¹²⁰ No significant differences in pulmonary hemodynamics and 6MWD were seen between the 2 groups at the end of 12 weeks. Pulmonary edema and pleural effusions occurred in 1 patient treated with fasudil resulting in death, and a causal relationship with the drug could not be excluded.

Statins

The cholesterol-independent pleiotropic properties of statins include antiproliferative, anti-inflammatory, and antithrombotic effects. In addition, statins indirectly block the posttranslational isoprenylation and activation of Rho and Ras small GTPases,¹²¹ increase endothelial NO production,¹²² and also enhance the expression of BMPR-II in experimental PH.¹²³

Statin therapy for PAH has been evaluated in 2 RCTs to date. In the Simvastatin for Pulmonary Hypertension Trial (SiPHT trial),¹²⁴ simvastatin therapy was associated with a small but significant reduction in right ventricular mass at 6 months, but this effect was not sustained at 12 months. No differences in exercise capacity or hemodynamics were observed in comparison with placebo. The Aspirin and Simvastatin for Pulmonary Arterial Hypertension (ASA-STAT) trial⁴³ used a 2x2 factorial design to assess the efficacy of aspirin and

simvastatin in PAH. The trial was stopped early after recruitment of 65 subjects when interim analysis suggested futility in reaching the primary end point of 6MWD for simvastatin.

Apelin

Apelin is an endogenous peptide with vasodilator effects on the pulmonary vasculature and its vasodilator property is, in part, related to the regulation of endothelial NOS levels.¹²⁵ Importantly, apelin has been identified as a key downstream target of BMPR-II signaling. Apelin expression is controlled by BMPR-II signaling mediated by a complex between β -catenin and peroxisome proliferator-activated receptor gamma.¹²⁶ Early-phase trials of apelin are underway in PAH (NCT01457170).

Inflammation in PAH

A link between inflammation and PAH pathogenesis can be supported by numerous observations including (1) histological evidence of infiltration of perivascular inflammatory cells in remodeled arteries, (2) the presence of autoantibodies against fibroblast, smooth muscle, and endothelial cells in a significant proportion of IPAH patients,^{127,128} (3) a close link between autoimmune diseases, HIV, and schistosomiasis infection in PAH, and (4) elevated serum levels of proinflammatory cytokines (such as interleukin-1 β , interleukin-6), and chemokines (such as regulated on activation, normal T cell expressed and secreted, monocyte chemoattractant protein-1, fractalkine) in PAH.¹²⁹⁻¹³¹ Recently, fully developed perivascular tertiary lymphoid follicles have been identified in IPAH lungs and are connected to remodeled vessels via stromal networks, suggesting that local adaptive immune response might be relevant in pathogenesis.¹³² Macrophage migration-inhibitory factor is a cytokine that simulates the release of proinflammatory mediators and administration of recombinant macrophage migration-inhibitory factor induces release of interleukin-6 and monocyte chemoattractant protein-1 from cultured human pulmonary endothelial cells. Macrophage migration-inhibitory factor are elevated in serum from PAH patients and CD-74 (receptor for macrophage migration-inhibitory factor) is highly expressed in endothelial cells from remodeled pulmonary arteries.¹³³

Anti-inflammatory therapies have been explored predominantly in connective tissue disorder-PAH, and small case series have reported the clinical response to immunosuppression by using a combination of cyclophosphamide with glucocorticoids. However, beneficial effects appear restricted to PAH associated with lupus and mixed connective tissue disorders but not in systemic sclerosis-associated PAH.¹³⁴ A phase II study investigating B-cell depletion with rituximab (monoclonal antibody against CD20) in scleroderma-PAH is underway (NCT01086540).

Mitochondrial Abnormalities

Mitochondrial abnormalities with a metabolic switch favoring cytosolic glycolysis rather than normal mitochondrial aerobic metabolism have been demonstrated in PAH. This switch to glycolysis for ATP synthesis, called the Warburg effect, was originally described in cancer cells. Although dependence on

glycolysis was initially thought to be a secondary effect of mitochondrial damage in cancer cells, there is evidence to support that enhanced glycolysis supports the metabolic requirements for cellular proliferation and confers resistance to apoptosis. PAH patients have increased lung fluorine-18–labeled 2-fluoro-2-deoxyglucose uptake in positron emission tomography, and hyperproliferative pulmonary artery fibroblasts isolated from IPAH patients exhibit upregulated glycolytic gene expression.¹³⁵

This metabolic abnormality can be partially corrected by the agent dichloroacetate, which inhibits pyruvate dehydrogenase kinase, the enzyme that phosphorylates and inactivates pyruvate dehydrogenase. Restitution of pyruvate dehydrogenase activity enables the irreversible oxidation of pyruvate to acetyl-CoA, which can then enter the Krebs cycle to initiate normal mitochondrial aerobic metabolism. Dichloroacetate has also been shown to reverse vascular remodeling in animal models of PH by increasing the mitochondria-dependent apoptosis/proliferation ratio and upregulating Kv1.5 in smooth muscle cells.^{136,137} A phase I open-label trial is currently investigating dichloroacetate in PAH (NCT01083524).

Restoration of BMPR-II Signaling Axis

Germline mutations in *BMPR2* are the most common genetic cause of PAH and the finding that BMPR-II function is also reduced in sporadic PAH has led to intense interest in manipulating and restoring BMPR-II function as a therapeutic target in PAH. Current evidence suggests that activation of the BMPR-II pathway functions as a protective system for pulmonary vascular remodeling, counteracting the proliferative effects of other stimuli.

BMPR2 Gene Transfer

A recent study showed that gene transfer of *BMPR2* using adenovirus vector ameliorated both hypoxia and monocrotaline models of PH in rats.¹³⁸ Furthermore, transforming growth factor β –induced endothelial-to-mesenchymal transition in human pulmonary microvascular endothelial cells was partially attenuated by restoration of BMPR-II signaling. The clinical translation of gene therapy will depend on ongoing advances in vector technology that can deliver long-lasting transgene expression.

Ataluren

Mutations in the *BMPR2* gene include missense mutations, nonsense mutations, splice defects, deletions, and duplications, of which nonsense mutations account for approximately half of all mutations.¹³⁹ Nonsense mutations result in the generation of premature stop codons and nonsense transcripts, which are recognized and degraded via the nonsense-mediated mRNA decay pathway. Ataluren is a drug already in phase III clinical trials in cystic fibrosis that interacts with ribosomes to enable the readthrough of premature stop codons. A recent in vitro study showed that treatment with ataluren can restore BMPR-II expression in peripheral blood and lung vascular cells from patients carrying nonsense *BMPR-2* mutations.¹⁴⁰

Antagomirs

Micro-RNAs (miR) are small noncoding RNA molecules that regulate gene expression at the posttranscriptional level. It has

been identified that the miR-17/92 cluster (mainly miR-20a) negatively regulates BMPR-II expression through an interleukin-6–dependent STAT3 pathway.¹⁴¹ A novel class of chemically engineered oligonucleotides termed antagomirs can lead to the silencing of miR. In the hypoxia-induced mouse model of PH, treatment with the antagomir directed against miR-20a enhanced the expression BMPR-II in lung tissues and attenuated pulmonary vascular remodeling.¹⁴²

Tacrolimus

With the use of the transcriptional high-throughput luciferase reporter assay, the calcineurin-inhibitor FK506 (tacrolimus) was recently found to be a candidate compound that can upregulate BMPR-II signaling.¹⁴³ Normal signal transduction involves BMPR-II forming a heterodimer complex with sister type-1 BMP receptors. In the presence of ligand binding, BMPR-II phosphorylates the intracellular domain of type-1 receptors, leading to activation of cytosolic Smads signaling cascade.¹⁶ FKBP12 is a molecule that interacts with type-1 BMP receptors and blocks its activation by occupying the phosphorylation site at its glycine-serine-rich region.¹⁴⁴ FK506 binds to and removes FKBP12 from type-1 BMP receptors, restoring normal signaling.¹⁴³ Low-dose FK506 also reverses both monocrotaline-induced and hypoxia/Sugen-induced rat models of PH.

Modulation of Intracellular Calcium

The increase in free cytosolic Ca²⁺ not only leads to PASM contraction, but also enhances proliferation via stimulation of Ca²⁺–dependent gene transcription.¹⁴⁵ Free cytosolic Ca²⁺ is regulated in PASM by voltage-dependent influx pathways (such as L- and T-type Ca²⁺ channels) or voltage-independent receptor- and stored-operated Ca²⁺ pathways (such as transient receptor potential channels¹⁴⁶ and Orai channels).^{147,148} Cytosolic Ca²⁺ homeostasis is also modulated by the sarcoplasmic reticulum Ca²⁺-ATPase2a (SERCA2a) which sequesters Ca²⁺ back into the sarcoplasmic reticulum/endoplasmic reticulum.

Kv channels, which control membrane potential and hence voltage-dependent Ca²⁺ influx, are downregulated in PASM from IPAH patients,¹⁴⁹ whereas store-operated Ca²⁺ channels (transient receptor potential channels and Orai channels) are upregulated.¹⁴⁷ More recently, SERCA2a protein expression was found to be downregulated in IPAH pulmonary arteries, and gene transfer of SERCA2a using aerosolized adeno-associated virus serotype 1 reversed pulmonary artery remodeling in monocrotaline-treated rats.¹⁵⁰ Manipulation of Ca²⁺ signaling pathways (other than the currently used voltage-dependent Ca²⁺ channels blockers) is another potential therapeutic target in PAH.

Progenitor Cell Therapies

Endothelial progenitor cells (EPCs) are bone marrow–derived progenitor cells believed to be involved in vascular homeostasis, and they can circulate, proliferate, and differentiate into mature endothelial cells at sites of vascular injury. Circulating EPCs appear both qualitatively and quantitatively altered in PAH,^{151–153} but their exact role in pathogenesis remains unclear. A human study of autologous transplantation of ex

Table 2. Characteristics of Phase III RCTs with Patients Exposed to Sequential Combination Therapy of Approved PAH Drugs

Drug Tested	Etiology	Background Therapy (%)	Monotherapy Arm	No. of Subjects	Duration	End Points		Comment	Ref
						Primary	Secondary		
Inhaled Iloprost (STEP)	IPAH, APAH	Bosentan	No	67	12 wk	6MWD	TTCW, PVR, FC, Borg score	Primary end point did not meet defined statistical significance ($P=0.051$). Improvement in TTCW, FC, and hemodynamics.	172
Inhaled Iloprost (COMBI)	IPAH	Bosentan	No	40	12 wk	6MWD	TTCW, FC, Peak VO_2 , QoL	All primary and secondary end points were not met. Trial was stopped early after interim analysis revealed low likelihood of reaching primary end point.	173
Inhaled Treprostinil (TRIUMPH-1)	IPAH, APAH	Bosentan (70%) or Sildenafil (30%)	No	235	12 wk	6MWD	TTCW, FC, QoL	Primary end point met. However, no difference in TTCW but QoL improved in treprostinil group.	174
Oral Treprostinil (FREEDOM-C)	IPAH, APAH	ERA (30%) or PDE-5i (25%) or both (45%)	No	350	16 wk	6MWD	TTCW, Borg score	Primary end point not met. No difference in TTCW.	58
Oral Treprostinil (FREEDOM-C2)	IPAH, APAH	ERA (17%) or PDE-5i (43%) or both (40%)	No	310	16 wk	6MWD	TTCW, FC, NT-proBNP, QoL	Primary end point not met. No significant differences in all secondary end points.	59
Tadalafil (PHIRST)	IPAH, APAH	Bosentan	Yes	216 (405)*	16 wk	6MWD	TTCW, PVR, QoL	Primary end point was met for entire study cohort, but subgroup on background therapy did not demonstrate improvement in 6MWD or FC.	78
Sildenafil (PACES)	IPAH, APAH	Epoprostenol	No	267	16 wk	6MWD	TTCW, PVR, QoL	Primary end point met together with delayed TTCW, improvement in hemodynamics and QoL.	175
Riociguat (PATENT-1)	IPAH, APAH	ERA (13%) or prostanoid (87%)	Yes	222 (443)*	12 wk	6MWD	TTCW, FC, PVR, NT-proBNP, QoL	Primary end point met and efficacy also demonstrated in prespecified subgroup on background therapy. Improved TTCW, FC, and PVR.	82
Macitentan (SERAPHIN)	IPAH, APAH	PDE-5i (92%) or nonparenteral prostanoid (8%)	Yes	471 (742)*	Median exposure 115 wk	Composite End point (Mortality and Morbidity)	6MWD, FC, QoL	Primary end point met and efficacy also demonstrated in prespecified subgroup on background therapy. Liver function abnormalities not significantly different in treatment groups.	72

APAH indicates associated pulmonary arterial hypertension; ERA, endothelin receptor antagonist; FC, functional class; IPAH, idiopathic pulmonary arterial hypertension; 6MWD, 6-minute walk distance; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAH, pulmonary arterial hypertension; PDE-5i, phosphodiesterase type 5 inhibitor; PVR, pulmonary vascular resistance; QoL, quality of life; RCT, randomized controlled trial; and TTCW, time to clinical worsening.

*Trial total.

vivo cultured EPCs has been performed in 31 PAH patients. The EPC-treated group displayed a significant beneficial treatment effect of +42.5 m ($P<0.001$) in 6MWD at 12 weeks.¹⁵⁴ Since this initial proof-of-concept study, no further human trials in adults have been reported.

An alternative stem cell type, mesenchymal stem cells, has potential advantages over EPCs given its greater ability to expand in culture and is considered immunologically privileged to allow allogeneic transplantation.¹⁵⁵ Mesenchymal stem cell-based gene therapies with overexpression endothelial NOS and PGI₂ synthase have both been shown to ameliorate rat models of PH.^{156,157} The safety and efficacy of mesenchymal stem cell transplantation in humans have not been reported to date. An improved understanding of the homing, engraftment, and paracrine effects of stem cells in injured pulmonary vessels are needed for therapeutic application to progress.

Neurohormonal Activation

Similar to congestive cardiac failure, PAH is characterized by neurohormonal activation as evidenced by increased sympathetic nerve traffic¹⁵⁸ and upregulation of the renin-angiotensin-aldosterone system. Plasma levels of renin, angiotensin I, and angiotensin II are increased in PAH and appear to correlate with worse prognosis.¹⁵⁹ β -Blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and aldosterone antagonists have all been demonstrated to confer beneficial hemodynamic effects in animal models of PH.¹⁶⁰⁻¹⁶⁴ In clinical practice, aldosterone antagonists are already widely prescribed in the treatment of right ventricular failure. In contrast, there are no data to support the use of β -blockers in PAH, and extreme caution is advocated owing to reports of symptom worsening with β -blocker administration.^{165,166}

Current Therapeutic Paradigm in Clinical Practice

The therapeutic approach in PAH has undergone significant evolution with the availability of multiple classes of drugs targeting different pathogenic pathways. It has also become apparent that therapeutic response with a single agent in patients with advanced disease in NYHA FC III/IV is frequently unsatisfactory. Therefore, the current treatment paradigm has shifted to an increasingly proactive approach, with emphasis on early disease intervention and the use of combination therapy. The use of combination therapy to achieve predefined treatment goals has emerged as a major theme to improve long-term outcomes in PAH.

Early Disease Detection

Symptoms are often nonspecific in early PAH, and exercise intolerance is often attributed to comorbid obesity, mild airflow limitation, or musculoskeletal disease. Specific populations at high risk for developing PAH (such as patients with systemic sclerosis and carriers of *BMPR-2* mutation) offer the opportunity for systematic screening and early disease intervention. There is compelling evidence supporting the superior survival of patients diagnosed in NYHA FC I to II in comparison with higher FC.^{167,168} A case-control study in the systemic sclerosis population has shown that patients diagnosed through a screening program in comparison with routine practice displayed lower NYHA FC, milder hemodynamic impairment, and better long-term survival.¹⁶⁹ An evidence-based screening algorithm for the early detection of PAH in systemic sclerosis (DETECT study) has recently been developed to guide referral for right heart catheterization based on the results of non-invasive evaluation.¹⁷⁰

Sequential Combination Therapy

Combination therapy with agents targeting different pathways is supported by a strong biological rationale and is an increasingly used strategy in PAH. The North American REVEAL registry indicated that 52% of patients were already on combination therapy in 2006 to 2007,¹⁷¹ and the prevalence of combination therapy in current clinical practice is likely to be even higher. However, the primary end point of combination therapy RCTs has not consistently been met, but a growing body of data is being accumulated supporting the beneficial effects of combination therapy (Table 2).^{58,59,72,78,82,172–176} The PACES trial demonstrated that the addition of sildenafil to background IV epoprostenol therapy improved 6MWD and delayed time to clinical worsening,¹⁷⁵ but this treatment sequence does not often correspond to clinical practice because oral therapy is usually initiated first. The 2 recently completed RCTs of macitentan (SERAPHIN trial)⁷² and riociguat (PATENT-1 trial)⁸² have substantially contributed to the evidence base of combination therapy. In both of these RCTs, at least 50% of enrolled subjects were on background therapy with predominantly oral PAH agents. The addition of macitentan 10 mg daily to patients on background therapy with either a PDE-5 inhibitor or nonparenteral prostanoid was associated with hazard ratio reduction versus placebo of 0.62 (95% confidence interval,

Table 3. Clinical Parameters Used in Treat-to-Target Strategy in PAH

Parameter	Target
NYHA FC	I or II
Echocardiography/CMRI	Normal/near-normal RV size and function
Hemodynamics	Normalization of RV function (RAP <8 mm Hg and CI >2.5–3.0 L·min ⁻¹ ·m ⁻²)
6MWD	>380–440 m; may not be aggressive enough in young individuals
Cardiopulmonary exercise testing	Peak $\dot{V}O_2$ >15 mL·min ⁻¹ ·kg ⁻¹ and $\dot{V}E_{CO_2}$ <45 L/min/L/min
B-type natriuretic peptide level	Normal

6MWD indicates 6-minute walk distance; CI, cardiac index; CMRI, cardiac magnetic resonance imaging; $\dot{V}E_{CO_2}$, ventilatory equivalent for carbon monoxide; NYHA FC, New York Heart Association functional class; RAP, right atrial pressure; RV, right ventricle; and $\dot{V}O_2$, oxygen consumption.

Adapted from McLaughlin et al¹⁷⁷ with permission from the publisher. Copyright © 2013, *Journal of the American College of Cardiology*.

0.43–0.89) for the composite morbidity and mortality primary end point. Similarly, riociguat improved 6MWD by 34 m (95% confidence interval, 11–56 m) in comparison with placebo in patients pretreated with either an ET-1 receptor antagonist or nonintravenous prostanoid. With the increasing number of approved PAH drugs, it remains unknown whether specific drug combinations provide superior efficacy, and no head-to-head data comparing different drug combinations are available.

Satisfactory treatment response not only implies disease stability but also the attainment of predefined treatment goals that places the patient into a better prognostic category. A number of invasive, and noninvasive parameters, as well, can be used to determine treatment response and the need to escalate therapy with additional PAH drugs (Table 3).¹⁷⁷ Treatment goals are based on parameters that confer prognostic significance,

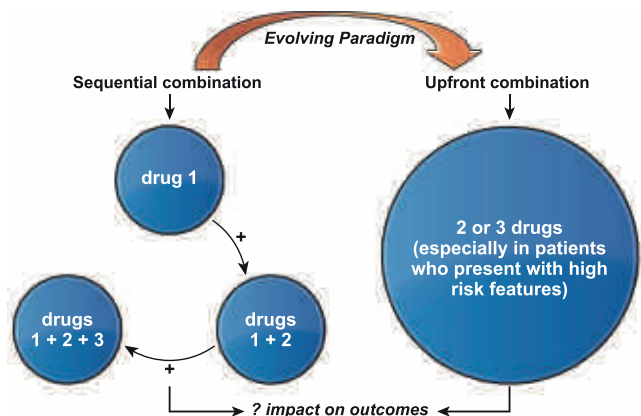


Figure 4. Paradigm of combination therapy in PAH. Combination therapy can be given by either sequentially adding agents if treatment response is unsatisfactory or by upfront combination. The traditional approach is sequential combination therapy in order to achieve treatment goals. However, there is emerging evidence to support the aggressive use of upfront combination therapy. At present, the ideal combination(s) of drugs (in terms of efficacy and safety) have not been fully determined. PAH indicates pulmonary arterial hypertension.

and the use of multiple parameters enhances the prediction of prognosis.¹⁷⁸ The use of goal-directed sequential combination therapy has been shown to provide improved long-term results with better survival rates and reduction in the need for lung transplantation in comparison with historical controls.¹⁷⁹ However, it is apparent that many patients will fall within a gray zone with clinical characteristics that do not necessarily place them into either a good or poor prognostic category; the best therapeutic strategy can be difficult to determine in this situation. The use of sequential combination therapy

together with treat-to-target strategy have been incorporated into the current evidence-based treatment algorithm in PAH (Figure 4).¹⁸⁰

Upfront Combination Therapy

In contrast to sequential combination therapy, only 1 published RCT has evaluated the use of upfront or initial combination therapy in PAH. BREATHE-2 was a small study of 33 PAH patients who were randomly assigned to upfront combination therapy with epoprostenol plus bosentan or epoprostenol

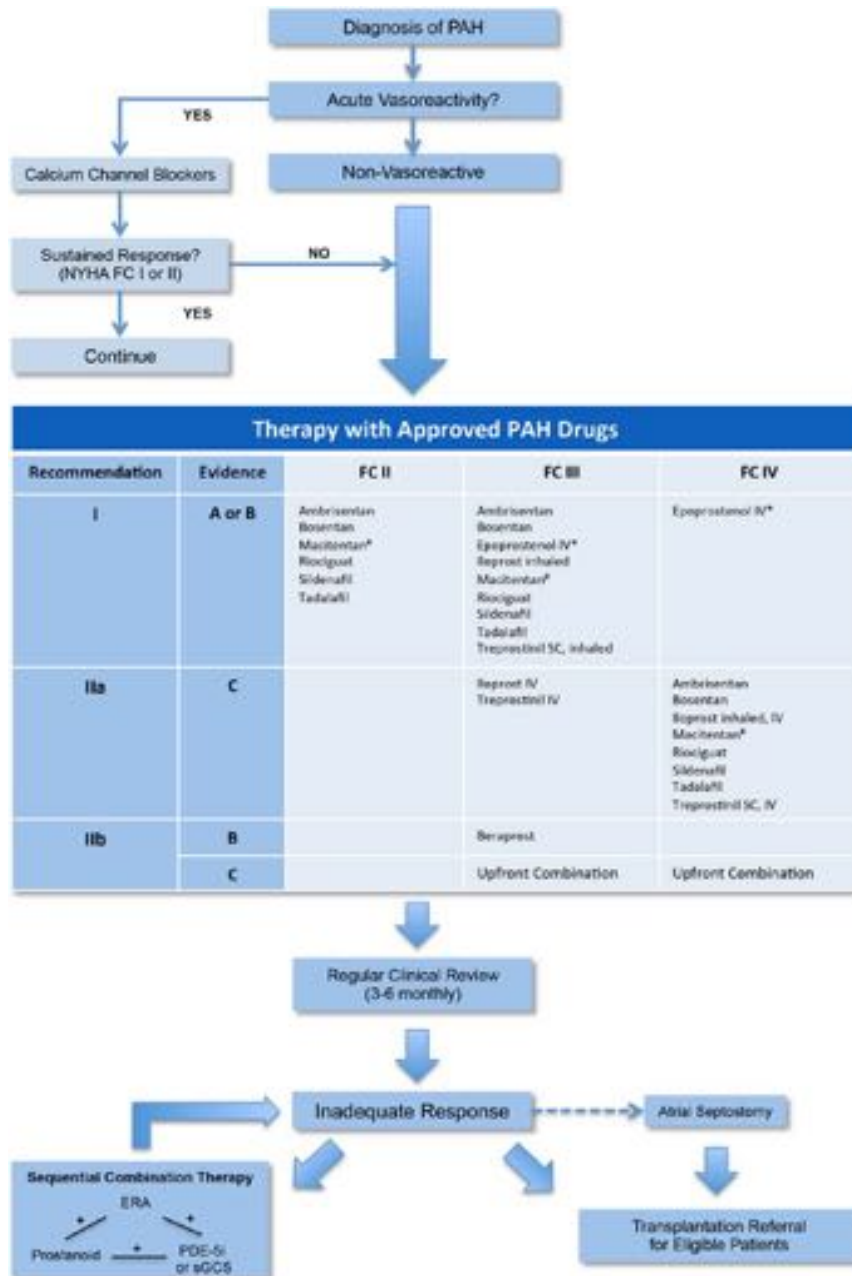


Figure 5. Evidence-based treatment algorithm in PAH. All PAH drugs have been approved on the basis of short-term efficacy by using 6MWD as the primary end point (with the exception of macitentan). *Epoprostenol demonstrated survival benefit in addition to improvement in 6MWD. #Macitentan was approved by using a composite morbidity and mortality primary end point in a long-term trial. BAS indicates balloon atrial septostomy; CCB, calcium channel blockers; ERA, endothelin receptor antagonists; FC, functional class; IV, intravenous; 6MWD, 6-minute walk distance; PAH, pulmonary arterial hypertension; PDE-5i, phosphodiesterase type-5 inhibitors; SC, subcutaneous; and sGCS, soluble guanylate cyclase stimulators. Adapted from Galiè et al¹⁸⁰ with permission from the publisher. Copyright © 2013, *Journal of the American College of Cardiology*.

alone.¹⁸¹ The primary end point of hemodynamic improvement was not met, although there was a trend toward a greater reduction in PVR in the combination therapy group. No significant differences in FC or 6MWD were observed, although this study was not powered to address these clinical end points.

The results of the Ambrisentan and Tadalafil in Patients with Pulmonary Arterial Hypertension (AMBITION) RCT have recently been released. In AMBITION, an upfront combination strategy with the combination of ambrisentan and tadalafil was compared with either ambrisentan or tadalafil alone, as first-line therapy in PAH. The combination of ambrisentan 10 mg and tadalafil 40 mg reduced the risk of clinical failure by 50 percent compared to the pooled ambrisentan and tadalafil monotherapy arm (hazard ratio = 0.50 (0.35 to 0.72); $p=0.0002$). The combination was also statistically significant versus the individual ambrisentan and tadalafil monotherapy groups for the primary endpoint ($p<0.01$). Rates of serious adverse events and events leading to discontinuation were similar across treatment arms. This study provides support for the emerging paradigm of upfront combination therapy and full results are expected to be published soon.^{181a}

A pilot observational study of upfront triple combination therapy with epoprostenol, bosentan, and sildenafil in patients with severe PAH who present in NYHA FC III/IV together with severe hemodynamic impairment was recently reported.¹⁸² Severe hemodynamic impairment was defined as either a cardiac index <2.0 L·min⁻¹·m⁻² or mean right atrial pressure >20 mm Hg or PVR >1000 dyn·s·cm⁻⁵. At a median follow-up of 39 months, 18 of 19 patients had sustained clinical improvement and remained in NYHA FC I/II. 6MWD increased dramatically from 227 to 514 m ($P<0.01$) and PVR declined from 1718 to 492 dyn·s·cm⁻⁵ ($P<0.01$). Despite the observational nature of this study, it provides preliminary proof of concept for the adoption of an aggressive upfront combination strategy in patients who have very severe PAH for whom prognosis has traditionally been extremely poor (Figure 5).

Nonmedical Therapies

Lung Transplantation

Lung transplantation remains a destination therapy for a significant number of patients despite targeted PAH therapy. Because of limited organ availability and the high mortality rates of PAH patients awaiting transplantation,¹⁸³ eligible patients for whom first-line treatment strategies have failed should be referred early for transplantation assessment. Double-lung transplantation is the preferred option for PAH, but heart-lung transplantation remains necessary for some patients in the context of complex Eisenmenger physiology and is also adopted by some centers for patients with refractory right heart failure.¹⁸⁴

Atrial Septostomy and Potts Shunt

Balloon atrial septostomy refers to the artificial creation of a right-to-left shunt to decompress the right ventricle.¹⁸⁵ This procedure can be accomplished percutaneously with careful graded balloon dilation and can improve peripheral oxygen delivery despite a fall in systemic arterial saturation because

of a compensatory rise in cardiac output.¹⁸⁶ This procedure has a high periprocedural mortality in patients with markedly elevated right atrial pressure¹⁸⁷ and should only be considered as a palliative therapy or bridge to transplantation in centers with experience in this procedure.

An alternative method of right ventricular decompression is via the creation of an anastomosis between the descending aorta and left pulmonary artery or Potts shunt. In the setting of suprasystemic PAP, a theoretical advantage of Potts shunt over septostomy is the sparing of the cerebral and coronary circulation from deoxygenated blood. A small case series of adults with NYHA FC IV PAH demonstrated the feasibility of the creation of Potts shunt via a minimally invasive percutaneous approach.¹⁸⁸ The safety and efficacy of this innovative technique require further study.

Pulmonary Artery Denervation

Borrowing the concept of renal artery denervation in the treatment of refractory systemic hypertension, pulmonary artery denervation is a novel nonmedical therapy for PAH and the presumed mechanism of action is via the abolishment of sympathetic nerve supply to the pulmonary circulation. In a first-in-human single-center study, 13 patients underwent pulmonary artery denervation with the use of a dedicated radio-frequency ablation catheter resulting in significant reduction of mean PAP (from 55 ± 5 mmHg to 36 ± 5 mmHg, $P<0.01$) and improvement in 6MWD (from 324 ± 21 m to 491 ± 38 m, $P<0.006$).¹⁸⁹ These preliminary results require further confirmation, and the negative findings of the recent Renal Denervation in Patients With Uncontrolled Hypertension (SYMPPLICITY HTN-3)¹⁹⁰ trial of renal denervation for resistant hypertension serves as a cautionary reminder that device-based therapies must also be subject to rigorous evaluation before adoption into clinical practice.

Conclusions

The past decade has witnessed major therapeutic advances in the treatment of PAH. Although current pharmacological agents have undoubtedly revolutionized the treatment landscape of this devastating condition, PAH remains a disease without a cure. By elucidating novel mechanisms and new signaling pathways in the pathobiology of PAH, one can expect to see further progress toward a cure in the exciting times ahead.

Disclosures

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