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Advancements In pulmonary arterial hypertension treatment

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**ADVANCEMENTS IN PULMONARY ARTERIAL HYPERTENSION
TREATMENT**

by

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ABSTRACT

Pulmonary arterial hypertension is a rare, chronic disease characterized by progressive remodeling of the pulmonary vasculature. Historically, prognosis has been very poor with relatively low 3-year survival rates. Common symptoms include fatigue and shortness of breath upon exercise, chest pain, and syncope. Patients exhibit increased pressure and resistance in pulmonary arteries due to fibrosis, vessel narrowing, and elevated levels of vasoconstrictive agents; diagnosis is confirmed by right heart catheterization. Reduced blood flow through the pulmonary vasculature not only reduces the amount of oxygenated blood available for the systemic circulation, but increases afterload on the right ventricle and, if left untreated, ultimately causes right ventricular heart failure.

In the past, few medications were available to pulmonary arterial hypertension patients. However, recent advancements in our molecular understanding of the disease have led to the development of new therapeutic options that show promise of slowing, or in some cases reversing, disease progression. Currently available treatments have been shown to significantly improve 3-year survival rates and help promote a better quality of life for patients. While an exact molecular or genetic mechanism of disease progression is not yet known, several studies have noted the presence of dysfunctional endothelial cells and an imbalance in molecular modulators of the pulmonary vasculature. Specifically,

patients exhibit chronically low levels of vasodilating agents such as prostacyclin and nitric oxide. In addition, there is a heightened vasoconstrictive effect due to elevated endothelin-1 and thromboxane A2. Drugs have been developed to target these signaling pathways and show considerable promise and efficacy for managing pulmonary hypertension in patients. Although these therapeutics have been shown to significantly improve survival rates and symptoms, many have complex and inconvenient administration protocols and a host of adverse side effects. Moreover, many require monitoring or frequent follow up visits due to their off-target effects. Recent innovative advancements in pulmonary arterial hypertension pharmaceuticals hope to deliver safe, efficacious treatment options to patients debilitated by this chronic disease.

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LIST OF ABBREVIATIONS

APAH.....	pulmonary arterial hypertension associated with other diseases
BMP	bone morphogenic protein
BMPR2	bone morphogenic protein receptor II
CCBs.....	calcium channel blockers
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
ET-1	endothelin-1
ETA.....	endothelin receptor A
ETB.....	endothelin receptor B
FPAH	familial pulmonary arterial hypertension
GPCR	G-protein coupled receptor
IAP	inhibitor of apoptosis
INR.....	international normalized ratio
IPAH	idiopathic pulmonary arterial hypertension
NO.....	nitric oxide
PAH	pulmonary arterial hypertension
PDE-5.....	phosphodiesterase-5
PDE-5i.....	phosphodiesterase-5 inhibitors
PDGF	platelet-derived growth factor
PGI2	prostacyclin

Introduction

Pulmonary arterial hypertension (PAH) is a rare progressive disease with an unfortunately poor prognosis. It is a subtype of pulmonary hypertension (PH) impacting the pre-capillary vasculature; the WHO has defined several groups of PH based on etiology (see Table 1). Typically, PAH has an incidence of 2 cases per million, with Idiopathic PAH (formerly named Primary PAH) representing the most common case (Humbert et al., 2006; Humbert and Lynch, 2009). The disease is characterized by increased pressure in the pulmonary arterial system (mean pulmonary artery pressure greater than 25 mmHg, at rest (Barst et al., 2004)) associated with vasoconstrictive vascular remodeling, thrombosis in situ, and proliferation of both arterial smooth muscle cells and endothelial cells (Farber and Loscalzo, 2004). Increased resistance in the pulmonary system ultimately leads to right ventricular heart failure and death; the NIH found a median survival rate of 2.8 years following diagnosis, and more recent studies have found an untreated 3-year survival rate of only ~67% (Humbert et al., 2010; Benza et al., 2012). Several factors have been found that positively correlate with a poor prognosis: age above 50, male gender, history of right ventricular dysfunction, decreased vasculature capacitance, WHO functional class 3 or 4, hypocapnia, elevated serum N-terminal brain natriuretic peptide, and others (Sitbon et al., 2002; Mahapatra et al., 2006; Fijalkowska et al., 2006; Benza et al., 2010).

Historically classified as either primary or secondary pulmonary hypertension, multiple classifications of PAH have now been established by the WHO: Idiopathic PAH

(IPAH), Familial PAH (FPAH), PAH associated with other diseases (APAH), etc (Table 2). Functional classes have also been established to assist physicians with diagnostics and treatment options (Table 3). Interestingly, the disease has a predominance towards women with a female to male ratio as high as 4:1 (Badesch et al., 2010). Mortality rates have been found to be higher in adult men, indicating that there may be an underlying hormonal component to disease progression (Austin et al., 2009; Mair et al., 2014).

Therapies for PAH have been developed to target molecules known to modulate pulmonary vasculature pathways; however, drug delivery methods remain inconvenient for patients and systemic side effects may cause adverse reactions. Prostacyclin (PGI₂), endothelin-1 (ET-1), and nitric oxide (NO) pathways (Figure 1) have been of particular interest for pharmaceutical companies (Sitbon and Morrell, 2012), but abnormal levels of thromboxane A₂, serotonin, and vasoactive intestinal peptide have also been found in PAH patients (Christman et al., 1992; Hervé et al., 1995; Petkov et al., 2003). PGI₂ asserts its vascular effects through activation of a G-protein coupled receptor (GPCR), the IP receptor, promoting vasodilation and inhibiting vascular smooth muscle proliferation (Lang and Gaine, 2015). PAH patients commonly have reduced prostacyclin synthase expression in pulmonary arteries (Tuder et al., 1999) and overall reduced PGI₂ levels (McLaughlin and McGoon, 2006), making this pathway a rationale target in terms of therapeutics.

Table 1. Classification of Pulmonary Hypertension by the World Health Organization (WHO). Types and subtypes of PH as defined during the Fifth World Symposium on Pulmonary Hypertension, 2013. Adapted from Simonneau et al., 2013.

Group #	Description and subtype
Group 1	Pulmonary arterial hypertension.
Group 2	Pulmonary hypertension caused by left heart disease. 2.1 Left ventricular systolic dysfunction 2.2 Left ventricular diastolic dysfunction 2.3 Valvular disease 2.4 Congenital/acquired left heart flow tract obstruction and congenital cardiomyopathies
Group 3	Pulmonary hypertension due to chronic lung disease/hypoxia. 3.1 Chronic obstructive pulmonary disease 3.2 Interstitial lung disease 3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern 3.4 Sleep-disordered breathing 3.5 Alveolar hypoventilation disorders 3.6 Chronic exposure to high altitude 3.7 Developmental lung diseases
Group 4	Pulmonary hypertension due to chronic thromboembolic pulmonary hypertension.
Group 5	Pulmonary hypertension due to unclear multifactorial mechanisms. 5.1 Hematologic disorders 5.2 Systemic disorders 5.3 Metabolic disorders 5.4 Others

Table 2. Types and Subtypes of Pulmonary Arterial Hypertension. Classification of PAH as defined during the Fifth World Symposium on Pulmonary Hypertension, 2013. Adapted from Simonneau et al., 2013.

Type	Subtype*
1.1 Idiopathic PAH	
1.2 Heritable PAH	1.2.1 BMPR2 1.2.2 ALK-1, ENG, SMAD9, CAV1, KCNK3 1.2.3 Unknown
1.3 Drug and toxin induced PAH	
1.4 PAH associated with other diseases	1.4.1 Connective tissue disease 1.4.2 HIV infection 1.4.3 Portal hypertension 1.4.4 Congenital heart diseases 1.4.5 Schistosomiasis
1' Pulmonary veno-occlusive disease/ pulmonary capillary hemangiomatosis	
1'' Persistent pulmonary hypertension of the newborn	

**BMPR* = bone morphogenic protein receptor type II; *CAV1* = caveolin-1; *ENG* = endoglin; *HIV* = human immunodeficiency virus.

Table 3. WHO Functional Classes for Pulmonary Hypertension. Clinical classifications for severity of PAH. Adapted from Galiè et al., 2009.

Class	Description
Class 1	Patients with PH but without resulting limitations of physical activity.
Class 2	Patients with PH resulting in slight limitations of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms to appear.
Class 3	Patients with PH resulting in marked limitations of physical activity. Comfortable at rest, but less than ordinary activity causes symptoms to appear.
Class 4	Patients with PH resulting in an inability to carry on physical activity without the occurrence of symptoms. These patients routinely develop signs of right heart failure. Dyspnea and fatigue may present at rest.

Similarly to PGI₂, NO is a vasodilator which inhibits vascular smooth muscle proliferation and platelet aggregation (McLaughlin and McGoan, 2006). Multiple points of intervention are targeted in this pathway: local endothelial cell production of NO increases levels of the intracellular secondary messenger cyclic guanosine monophosphate (cGMP) and the signal is terminated in pulmonary tissue by phosphodiesterase-5 (PDE-5) enzymatically hydrolyzing cGMP. Therapeutics have been

developed both to increase cGMP levels and inhibit PDE-5 activity (Lang and Gaine, 2015). In contrast to PGI₂ and NO, ET-1 levels are elevated in PAH patients and may be positively correlated with poor prognosis (Rubens et al., 2001). ET-1 has vasoconstrictive and proliferative effects on endothelium and vascular smooth muscle, and many ET-1 antagonists have been developed as therapeutics (McLaughlin and McGoon, 2006). Adverse effects of traditional ET-1 receptor antagonists, particularly hepatotoxicity (McGoon et al., 2009), require physicians to monitor patients and carefully consider the safety profiles of drugs. Over the last decade, many exciting advancements have been made in the field of pulmonary hypertension treatment, and hopes are high for improving prognosis for patients. In particular, innovations in drug delivery methods and reduction of adverse side effects may help with patient adherence and physician utilization, as most commonly used therapeutics are not currently available in oral formulation (Lang and Gaine, 2015).

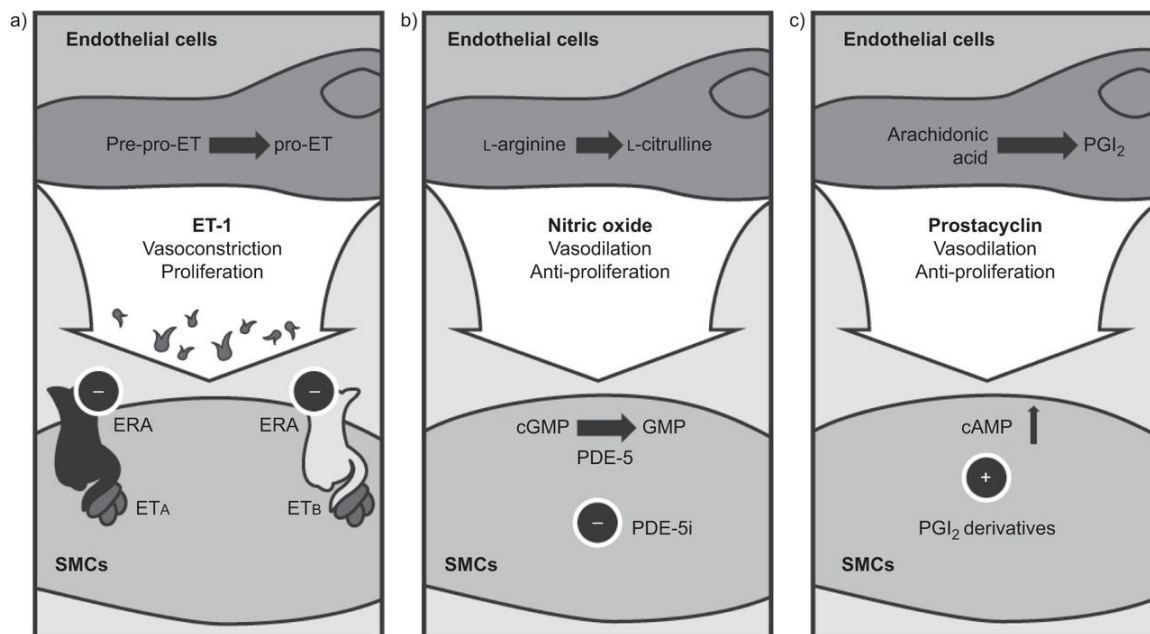


Figure 1: Major pathways targeted for therapeutic intervention in pulmonary arterial hypertension disease progression. From left to right: endothelin-1 (ET-1) pathway; nitric oxide (NO) pathway; prostacyclin (PGI₂) pathway. Figure taken from Sitbon and Morrell, 2012. *ERA* = *ET receptor antagonist*; *cGMP* = *cyclic guanosine mono phosphate*; *PDE-5i* = *phosphodiesterase-5 inhibitor*; *cAMP* = *cyclic adenosine mono phosphate*; *SMCs* = *smooth muscle cells*.

Specific Aims

The goals of this paper are to provide a concise overview of the current literature defining disease pathology and currently utilized therapeutics in pulmonary arterial hypertension. In addition, emerging treatment options and therapeutics currently in development will be discussed with emphasis on their potential for improving patient prognosis and quality of life. Finally, potential genetic mechanisms of therapeutic intervention will be briefly discussed as the future of PAH treatment.

Therapeutic Pathways in Pulmonary Arterial Hypertension

Compared to the systemic circulation, the pulmonary circulation is a low pressure system with thin arterial walls and low resistance. The pathobiology of PAH is characterized by the dysfunction of a variety of cell types: vascular smooth muscle cells, endothelial cells, fibroblasts, and platelets (Humbert et al., 2004), leading to vascular remodeling and narrowing of blood vessels. Notably, increased proliferation of smooth muscle cells is accompanied by their migration to more distal arteries which do not normally have musculature in healthy patients (Humbert et al., 2004). Combined with inappropriate levels of vasomodulating molecules causing increased vasoconstriction, pressure in the pulmonary system rises well above normal values. The molecular mechanism by which smooth muscle cells migrate to smaller, more distal vessels is not entirely clear but may involve interactions between several signaling molecules.

Endothelial cell dysfunction is thought to be a principal factor in the progression of PAH (McLaughlin and McGoon, 2006). Not only is abnormal endothelial cell proliferation a major concern as it can cause severe vessel thickening and the formation of plexiform lesions (Humbert et al., 2004), there is an imbalance in the production of molecules modulating vasoconstriction, molecules modulating smooth muscle cell growth, and molecules modulating thrombosis and inflammation. An underlying genetic explanation may involve defective growth suppression genes, particularly the transforming growth factor beta type 2 receptor (TGF-BR2) gene (Yeager et al., 2001). Studies have shown that 90% of plexiform lesions found in the pulmonary vasculature have endothelial cells lacking TGF-BR2 expression (Humbert et al., 2004).

In addition to the TGF- β 2 mutation, many other possible genetic markers for PAH exist within the TGF- β family. Most notable is the bone morphogenetic protein receptor 2 (BMPR2), which forms part of a heteromeric serine/threonine kinase receptor found on vascular smooth muscle cells (Liu et al., 1995). Mutations in the gene encoding BMPR2 are heavily associated with cases of familial PAH, and it is believed faulty signal transduction leads to increased proliferation of smooth muscle cells in pulmonary vasculature (McLaughlin and McGoon, 2006). This represents a promising avenue for diagnostic markers, as mutations in the BMPR2 gene have been found in 60-80% of FPAH patients and in 10-40% of IPAH patients, with the majority of mutations causing a nonfunctional protein through premature termination (Humbert et al., 2004; Machado et al., 2006). Mutations in other receptors, such as the activin-like kinase type 1 receptor (Trembath et al., 2001), and increased expression of serotonin transporters (Eddahibi et al., 2001), are also implicated in PAH. However, genetic therapies have remained elusive in modern PAH management. The lack of BMPR2 mutations in the majority of IPAH cases may implicate that other genes, yet to be identified, are also responsible for the disease pathology.

The molecular mechanisms underpinning the progression of PAH have received a considerable amount of attention in recent years. In terms of therapeutic options, PAH patients have elevated levels of the vasoconstrictor ET-1, and reduced vasodilators NO and PGI₂. Modern therapeutic advances have focused primarily on these three major pathways.

Endothelin-1 pathway

Endothelin-1 is a 21 amino acid polypeptide released by endothelial cells with high levels of expression in the pulmonary endothelium (Chester and Yacoub, 2014). ET-1 levels are mostly regulated at the transcriptional level, as well as by post-translational cleavage of the proenzyme (Chester and Yacoub, 2014). These processes are regulated by many different factors: hypoxia, shear stress, and growth factors increase ET-1 production, while PGI₂, NO, and high estrogen act to decrease ET-1 levels (Galié et al., 2004). The effect of estrogen specifically may help explain the gender differences in PAH disease progression, and the inhibitory effects of PGI₂ and NO on ET-1 synthesis indicate a level of cross-talk between the vasomodulating pathways. Interestingly, ligands for BMPR2 have been shown to diminish the action of ET-1, suppressing contraction of smooth muscle cells upon the activation of the ET-1 receptor (Chester and Yacoub, 2014), indicating a possible interaction between BMPR2 mutations and ET-1 levels.

The ET-1 receptor itself is a GPCR found on vascular smooth muscle cells, activation of which triggers a phospholipase C-mediated signal cascade that ultimately increases levels of intracellular calcium (Pollock et al., 1995). Binding of ET-1 to its receptor on vascular smooth muscles cells causes activation of contractile machinery (Chester and Yacoub, 2014), increasing arterial pressure and contributing to the effects of pulmonary hypertension.

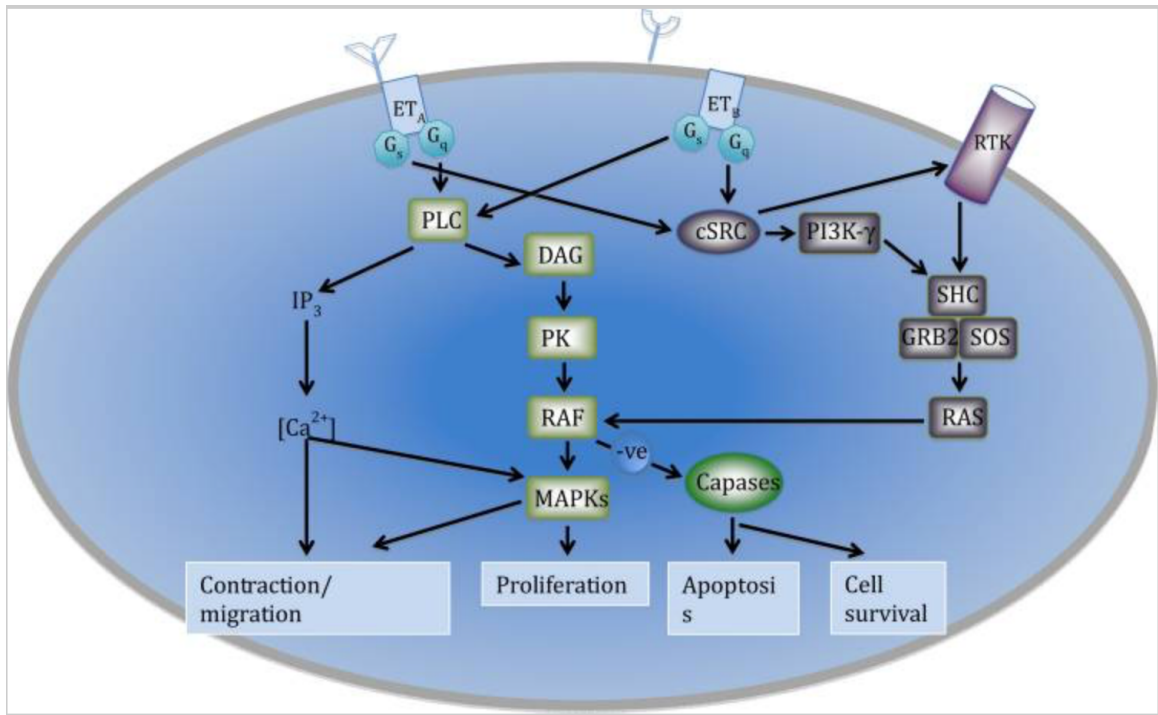


Figure 2: Endothelin-1 pathway signaling cascade linked to pulmonary vascular smooth muscle cell contraction, proliferation, and apoptosis. Activation of the ET-1 pathway causes contraction, cellular migration, and proliferation, while suppressing apoptosis. Figure taken from Chester and Yacoub, 2014. *ETA* = ET receptor A; *ETB* = ET receptor B; *PLC* = phospholipase C; *IP3* = inositol 1,4,5-triphosphate; *DAG* = diacylglycerol; *PK* = protein kinase C; *RAF* = rapidly accelerated fibrosarcoma protein; *MAPKs* = mitogen activated protein kinases; *cSRC* = cytosolic tyrosine kinase; *PI3K-γ* = phosphatidylinositol 3-kinase gamma; *SHC* = Src homology 2 domain-containing; *GRB2* = growth factor receptor-bound protein 2; *SOS* = son of sevenless protein; *RAS* = rat sarcoma protein; *RTK* = receptor tyrosine kinase.

The receptor also has a role in regulating smooth muscle proliferation and cell migration (Figure 2), contributing to the vascular remodeling seen in PAH. ET-1 has been extensively studied as an agent for PAH therapy: PAH patients have elevated levels of ET-1 due to both increased production and reduced clearance (Stewart et al., 1991).

Nitric Oxide pathway

NO is a potent vasodilator produced in pulmonary epithelium and vascular endothelial cells. It is synthesized by the enzymatic action of NO synthase, activity of which is regulated transcriptionally and by post-translational modifications (Michelakis, 2004). Local NO levels play an important role in the vasodilation of pulmonary vasculature and ventilation-perfusion matching, and may delay the progression of vascular remodeling seen in PAH (Sparacino-Watkins et al., 2012). Regulation of the NO pathway is rather complicated, involving secondary messenger signal cascades and the action of multiple enzymes, such as guanylate cyclase and phosphodiesterase (PDE) (Figure 3). Furthermore, these enzymes may have interactions with proteins in the TGF family (Kolosionek et al., 2009), indicating that there may be an underlying genetic dysfunction affecting the NO pathway in PAH patients. While there are currently 11 known PDE isoforms with varying tissue distribution, they all act to degrade cyclic mononucleotides (Barst, 2008) and terminate the signaling cascade initiated by substrates such as NO. Antagonizing generalized PDE action may impact other signaling pathways that rely on cGMP. In the lungs, PDE-5 is the primary isoform terminating the NO signal (Ghofrani et al., 2004), making inhibitors of this specific protein an attractive target for PAH therapy. While PDE-5 inhibitors (PDE-5i) have been shown to reduce the elevated pulmonary arterial pressure and vasoconstriction associated with hypoxia (Zhao et al., 2001), further studies are needed on their efficacy on reversing the adverse vascular remodeling seen in PAH.

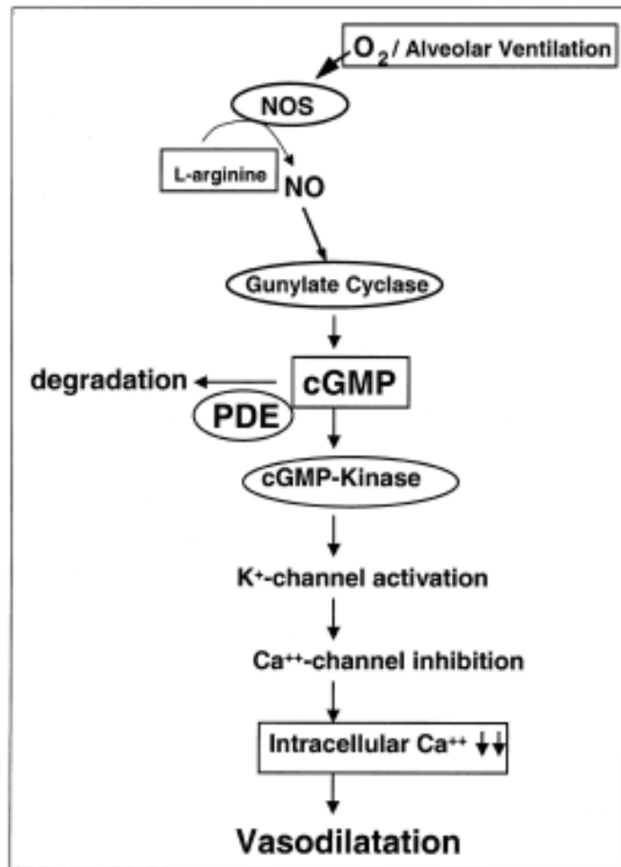


Figure 3: Nitric Oxide pathway signaling cascade linked to vasodilation of the pulmonary vasculature. Activation of the NO pathway by oxygen and alveolar ventilation decreases contractile force in pulmonary vascular smooth muscle cells. The signal is terminated by the action of phosphodiesterase. Figure taken from Ghofrani et al., 2004. *NOS = NO synthase; NO = nitric oxide; cGMP = cyclic guanylate monophosphate; PDE = phosphodiesterase.*

Added benefits of PDE-5 inhibitor therapy include a well documented safety profile and lack of some of the serious systemic side effects seen in other PAH medications.

Prostacyclin pathway

Prostacyclin, or prostaglandin I₂ (PGI₂), is produced by endothelial cells and acts by increasing intracellular levels of the secondary messenger adenosine monophosphate (cAMP) (McLaughlin and McGoon, 2006). Activation of the PGI₂ pathway causes vasodilation of the pulmonary vasculature, as well as decreased platelet aggregation and reduced proliferation of vascular smooth muscle cells (Humbert et al., 2004). PAH patients have been shown to have markedly reduced levels of both PGI₂ and prostacyclin synthase (Tuder et al., 1999), the enzyme responsible for producing PGI₂ from arachidonic acid (Figure 4). The PGI₂ receptor, or IP receptor, has been identified as a potential target for PAH therapy, but limitations of current therapeutics have caused widespread underutilization of prostanoid therapy (Farber et al., 2011). Higher levels of thromboxane A₂ are noted in PAH patients (Christman et al., 1992) and may be related to dysfunction in the PGI₂ synthesis pathway, contributing to the elevated pulmonary arterial pressure.

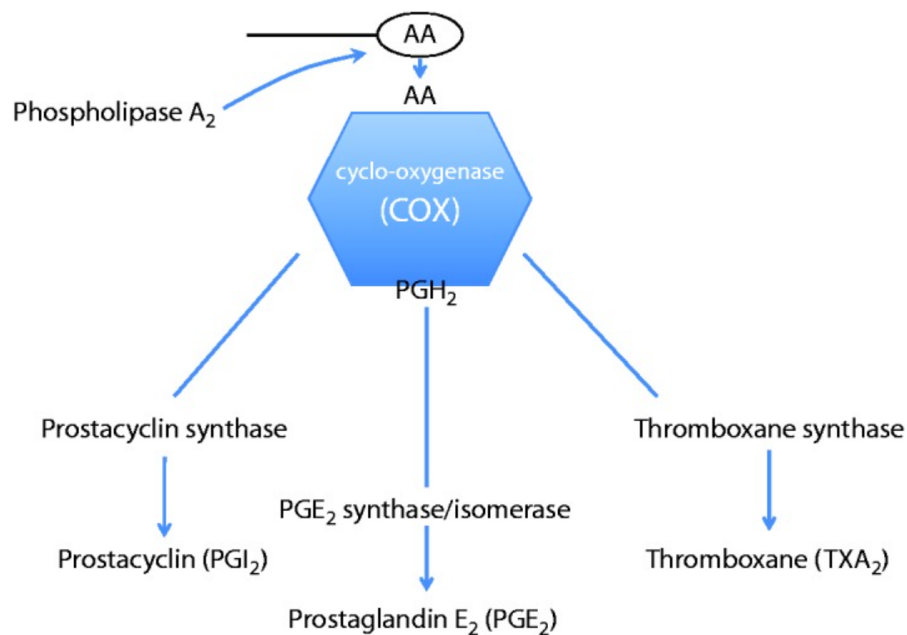


Figure 4: Synthesis pathway of prostacyclin. Membrane arachidonic acid is liberated by phospholipase A₂ and ultimately converted to prostacyclin in endothelial cells by enzymatic activity. Figure taken from Mitchell et al., 2015. *AA* = arachidonic acid; *PGH₂* = prostaglandin; *PGI₂* = prostacyclin; *PGE₂* = prostaglandin E₂.

Current Treatments

Lifestyle

Special care must be taken by PAH patients in daily activities due to their reduced effective pulmonary circulation. With that said, exercise should not be avoided altogether: a small study found that exercise and physical training had an overall positive effect on quality of life on PH patients (Mereles et al., 2006). Caution must be taken before strenuous exercise, as increased oxygen demand may cause life-threatening syncope (McLaughlin and McGoon, 2006); patients should cease activity if symptoms occur. In addition, patients with advanced PAH should avoid ascending to high altitudes

as it may cause hypoxia and subsequent worsening of pulmonary vasoconstriction (Moudgil et al., 2005). Supplemental oxygen therapy is indicated for patients with a mean arterial oxygen saturation below 90% (McLaughlin and McGoon, 2006), except in patients with Eisenmenger syndrome (Sandoval et al., 2001).

Female patients must take extreme caution during pregnancy, labor, and even the postpartum period. Maternal changes in hemodynamics and increased oxygen demand during pregnancy and childbirth can cause devastating and potentially fatal effects in PAH patients (Weiss et al., 1996). Even with modern advancements in treatment options, maternal mortality rates in women with PAH remain high, with most deaths occurring within the first month following delivery (Bédard et al., 2009). Although cases of successful deliveries in PAH patients on prostacyclin therapy (e.g. epoprostenol) have been reported (Bendayan et al., 2005), the general expert consensus is for pregnancy to be avoided and contraceptive measures taken.

Conventional therapy

Most studies use specific baseline characteristics to measure the efficacy of treatment. Improvements in exercise capacity are characterized by 6-minute walk distances, an inexpensive, safe clinical test that has been shown to be a strong prognostic marker in PAH (Miyamoto et al., 2000). Hemodynamic factors such as pulmonary arterial pressure and right ventricular function are also measured. As noted, a consequence of advanced PAH is right ventricular failure and subsequent volume overload. On this basis, diuretics have been employed to reduce blood volume and right

ventricular preload, offering notable improvements in patients with right heart failure (Humbert et al., 2004). Often a loop diuretic is first choice, usually furosemide or torsemide (Murray et al., 2001), or intravenous diuretics in the case of poor absorption (Barst, 2008).

While warfarin has been known to have a myriad of drug interactions (Holbrook et al., 2005; Spangler and Saxena, 2010), it has been indicated as an effective PAH therapy. Dysfunction in endothelial and platelet function leads to increased intravascular thrombosis in PAH patients, with related elevation of plasma clotting factors (Humbert et al., 2004). Thrombotic lesions are routinely found in pulmonary blood vessels of PAH patients (Moser et al., 1995; Hervé et al., 2001) and disease progression may be accelerated by platelet release of vasoconstrictors and vascular remodeling agents, such as thromboxane A2 and serotonin (Humbert et al., 2004). Unfortunately, the effectiveness of warfarin as a PAH therapeutic has been evaluated in few studies with relatively small sample sizes (Frank et al., 1997; Rich et al., 1992; Fuster et al., 1984). In all three, use of anticoagulants improved survival time in PAH patients, leading many experts to recommend the use of warfarin with a target international normalized ratio between 1.5 and 2.5 (Barst, 2008; Humbert et al., 2004; McLaughlin and McGoon, 2006). More research is needed to solidify guidelines for warfarin therapy, particularly when used in combination with modern drugs.

Another approach to PAH treatment has been the use of calcium channel blockers (CCBs). Vascular smooth muscle cells require an influx of calcium to initiate contraction, the rationale for CCB therapy involves the blocking calcium channels to reduce

vasoconstriction in pulmonary arteries. However, the use of CCBs in PAH treatment remains somewhat controversial. Not all patients react favorably, with one study showing that only half of IPAH patients responsive to CCBs benefited from long term therapy after a 5-year period (Sitbon et al., 2003). In addition, reactivity tests to acute vasodilators must be done to identify patients who may benefit from CCB therapy, requiring cardiac catheterization and intravenous administration of prostacyclin or inhalation of NO (Sitbon et al., 1995). Many patients do not respond to CCBs at all (i.e. chronic responders), and as such administration of CCBs may cause negative side effects without providing any benefit to the patient.

Prostacyclin therapy

While the treatments described above have been at the frontline of pulmonary hypertension treatment, recent and exciting advancements in the understanding of PAH have led to more targeted therapies. New therapeutics designed specifically to target the pulmonary arterial vasculature have shown considerable promise in decreasing side effects and mortality rates and ushering patients in a modern era of PAH treatment. Among these, therapeutics targeting the prostacyclin pathway have shown favorable outcomes in clinical trials. While new medications, with more favorable dosing regimens and routes of administration, targeting this pathway have been recently approved by the FDA, prostacyclin drugs for PAH patients have existed for some time.

Among the first prostacyclin-targeted drugs emerged in the 1980s, epoprostenol. Epoprostenol is a prostacyclin agonist which has antiproliferative, antithrombotic, and

vasodilator effects (McLaughlin et al., 1998). While this pharmaceutical has been shown to considerably improve patient function, especially in those unresponsive to conventional therapy, a major drawback of the medication is the risk for adverse complications, such as sepsis and thromboembolism (McLaughlin et al., 1998), due to its short half-life requiring continuous intravenous administration by a permanent venous catheter. Studies have shown that, compared to conventional therapy alone, epoprostenol can significantly improve patient exercise capacity, reduce pulmonary blood pressure and vascular resistance, and boost patient survival rates (Barst et al., 1996; Rubin et al., 1990). Historically, epoprostenol therapy made a substantial impact on PAH patient outcomes: one study found that 70% of patients were removed from a lung transplantation waiting list due to clinical improvements on intravenous epoprostenol monotherapy (Conte et al., 1998). Indeed, lack of alternative targeted therapies and considerable improvements over conventional therapies allowed intravenous epoprostenol to become a frontline therapy in advanced cases of PAH.

Treprostinil is a more stable prostacyclin agonist which can be administered via continuous subcutaneous infusion, avoiding the risks associated with intravenous epoprostenol administration. While most improvements were seen with high dosing regimens, placebo-controlled studies confirmed the benefits of treprostinil therapy on exercise capacity: 6-minute walk distances improved by a modest 16 m (Simonneau et al., 2002). However, 85% of patients reported pain at the infusion site and 8% terminated their treatment altogether (Simonneau et al., 2002). In other studies, as many as 23% of patients terminated treprostinil therapy due to adverse side effects (Barst et al., 2006).

Due to the frequency of pain at the subcutaneous infusion site, an intravenous treprostinil therapy was approved for PAH treatment in 2004. A longer half-life allowed intravenous treprostinil significant benefits over intravenous epoprostenol, such as stability at room temperature and a higher safety profile should infusion be abruptly interrupted (Gomberg-Maitland et al., 2005). While much higher doses were needed, i.v. treprostinil showed a similar efficacy when compared to epoprostenol (Gomberg-Maitland et al., 2005). Still, a therapeutic option convenient for out-patient PAH management remained elusive.

A novel therapy was approved by the FDA in 2004 which involved the oral inhalation of a long-acting prostacyclin analogue. Iloprost was developed to circumvent the adverse effects related to continuous infusion and increase selectivity for the pulmonary vasculature. It is administered via jet or ultrasonic nebulizers, but due to its relatively short half-life it must be given 6 to 9 times daily (Hoepfer et al., 2000). Studies found fewer systemic side effects and a longer duration of pulmonary vasodilation when compared to intravenous prostacyclins (Olschewski et al., 2002). Long term observational studies did show improvements to 6-minute walk distances of up to 75 m in some patients, and improvements to physical capacity and hemodynamics were sustained over 12 months (Hoepfer et al., 2000). Inhaled iloprost may be an inexpensive, safer option when compared to a permanent venous catheter, with few patients reporting mild adverse effects and no patients discontinuing use in clinical studies (Hoepfer et al., 2000).

Beraprost was the first oral prostacyclin therapy available and has vasodilating, antiproliferative, and antiplatelet effects similar to intravenous epoprostenol, but with a

much longer half-life (Nishio and Kurumatani, 2001). The prospect of a treatment option without continuous infusion or multiple daily inhalations was attractive to physicians and patients alike, as it would avoid many potential complications associated with conventional prostacyclin administration. Beraprost showed considerable improvement in managing acute disease progression, with patients showing an improvement in 6-minute walk distance of 25 m (Galiè et al., 2002). This effect, however, was not evident after 12 months, and patients did not show a slowed disease progression compared to placebo at 12 months (Barst et al., 2003). Beraprost may be an effective treatment in managing symptoms in early PAH, but its efficacy seems to decline over chronic use. Mild adverse effects, such as headaches, jaw pain, and nausea, were reported in the majority of patients, and 3% discontinued use due to these effects (Barst et al., 2003). These findings also emphasize the importance of the length of clinical studies of PAH treatments. Beraprost might have been inappropriately touted as a hugely beneficial drug had all clinical studies lasted only 3 months.

Endothelin antagonist therapy

Endothelin-1 has long been implicated in the progression of pulmonary hypertension, with PAH patients having significantly elevated plasma levels. ET-1 is thought to exert its hypertensive effects by acting on the ETA or ETB membrane receptors on pulmonary smooth muscle to promote vasoconstriction, proliferation and hypertrophy (Barst, 2008). It should be noted that activation of the ETB receptor on endothelial cells promotes vasodilation and overall clearance of ET-1 from vasculature

(Felix et al., 2003). The ultimate effects of ETB antagonism on PAH disease progression requires further study, as ETB receptors on endothelial cells may have a protective role (Yorikane et al., 1992; Nishida et al., 2004).

Bosentan is an orally available ET-1 antagonist at both the ETA and ETB receptors. It has been shown to increase 6-minute walk distances by 70 m at 12 weeks and reduce both pulmonary arterial pressure and resistance (Channick et al., 2001). Other studies found a more modest improvement to 6-minute walk distance of 44 m after 16 weeks (Rubin et al., 2002). While a benefit of bosentan therapy over epoprostenol is the avoidance of potentially complicated continuous infusion, combination therapy may be more efficacious than either therapy alone (Humbert et al., 2004). The drug carries the potential for many adverse effects, particularly hepatic toxicity and elevated levels aminotransferases (Rubin et al., 2002). The adverse effects on the liver are thought to be mediated through a drug-induced increase in cytotoxic bile salts (Fattinger et al., 2001). Patients must be monitored while receiving treatment with follow-up liver function and hematocrit tests. In addition, pregnancy becomes an issue with this therapy due not only to bosentan's teratogenic effects but its interaction with traditional hormone-based contraceptions (Dhillon, 2009), requiring women to use alternatives to avoid pregnancy.

Sitaxsentan is a selective ETA receptor antagonist given once daily. Due to its increased selectivity for the ETA receptor compared to bosentan, sitaxsentan was thought to be advantageous due to a lack of inhibition to the potentially protective endothelial cell ETB receptor pathway. However, a study found comparable improvements in exercise capacity between bosentan and sitaxsentan treatments, increasing 6-minute walk distance

by 29.5 m and 31.4 m, respectively (Barst et al., 2006). A long term study was able to show that patients on sitaxsentan had an 8% higher survival rate when compared to bosentan therapy over 1 year (Benza et al., 2008). In fact, a small sample study found that patients with elevated transaminases due to bosentan treatment were able to stabilize plasma transaminase levels by transitioning to sitaxsentan (Benza et al., 2007). Still, the safety profile for sitaxsentan was a concern for regulators and the drug was voluntarily withdrawn from clinical trials before reaching the US market due to fatalities related to liver damage (Galiè et al., 2011). Hepatic injury and elevated aminotransferase levels are a major concern with the endothelin-1 antagonist class of drugs (Barst et al., 2006).

Similar to sitaxsentan, ambrisentan is a once-daily oral ETA-selective endothelin antagonist. It shows a lower incidence of liver abnormalities, with one study showing that 261 patients receiving ambrisentan did not develop elevated aminotransferase levels (Galiè et al., 2008). While ambrisentan may be a safer alternative in terms of liver injury when compared to bosentan, it may cause clinical worsening in patients with pulmonary fibrosis (Raghu et al., 2013). In PAH patients, ambrisentan therapy was shown to increase 6-minute walk distance by 45 m over 12 weeks and significantly delay disease progression (Galiè et al., 2008). Improvements were sustained over long term treatment, with patients showing increases in 6-minute walk distances of 28 m and 23 m after 1 year and 2 years, respectively (Oudiz et al., 2009). In fact, most patients showed either an improvement or sustainment of their WHO functional class over 2 years of treatment (Oudiz et al., 2009), indicating this therapeutic may be beneficial with minimal adverse effects, particularly in combination therapy.

While these drugs represent a rational avenue for PAH treatment, targeting the endothelin-1 pathway has proved difficult in many ways. Notably, hepatotoxicity remains a major concern. Decreases in hematocrit (Barst, 2008) also require further investigation, along with interactions with the metabolism of warfarin (Walker et al., 2009). As noted, pregnancy can be fatal for some PAH patients and ET-1 antagonists may reduce the efficacy of certain contraceptives while also causing teratogenic harm in animal models (Barst, 2008). Physicians must carefully weigh the benefits and side effects before commencing treatment by this pathway, and monitor patients accordingly.

Phosphodiesterase-5 Inhibitors

Phosphodiesterase-5 inhibitors act by potentiating the effects of the NO pathway. NO itself is commonly used as an agent for vasoreactivity tests for consideration of CCB therapy. PDE genes are up-regulated in cases of pulmonary hypertension (Maclean et al., 1997). The rationale for PDE-5i agents in pulmonary hypertension therapy is to prolong the cGMP-mediated NO signaling pathway, effectively lowering intracellular calcium levels and reducing contraction of vascular smooth muscle cells.

Sildenafil was initially researched for the treatment of hypertension, but found resounding commercial success as the first oral therapy for erectile dysfunction. It acts as a selective PDE-5i, prolonging the effects of local NO produced by endothelial cells (Goldstein et al., 1998) allowing vasodilation and aiding in proper ventilation-perfusion matching in the lungs. Early studies showed that treatment with sildenafil was able to prevent pulmonary hypertension in hypoxic mice models (Zhao et al., 2001). Further

clinical trials were able to show an increase in 6-minute walk distance of 45m after 12 weeks of sildenafil, and significant improvements to both pulmonary arterial pressure and resistance (Galiè et al., 2005). Furthermore, a 12 month follow-up showed mild adverse side effects with sildenafil therapy, and continued, albeit modest, improvements to exercise capacity (Galiè et al., 2005). Combination therapy also shows considerable promise, as sildenafil combined with inhaled iloprost was found to significantly improve both hemodynamics and 6-minute walk distances in patients deteriorating on iloprost therapy alone (Ghofrani et al., 2003). In addition, while PAH in children is relatively rare, researchers were able to show a remarkable increase in 6-minute walk distances and a lowering of pulmonary artery pressure by 10 mmHg over 12 months (Humpl et al., 2005). This PDE-5i therapeutic seems to represent a safe, affordable therapy (Michelakis et al., 2003) with a more convenient route of administration compared to prostacyclin pharmaceuticals and without some of the adverse side effects related to targeting the ET-1 pathway.

Tadalafil is an alternative to sildenafil with the advantage of once-daily dosing, but only showing efficacy at higher doses (Galiè et al., 2009). Patients showed modest improvements in 6-minute walk distances over four months, and these effects were sustained over 52 weeks (Oudiz et al., 2012). While this treatment seemed to delay the worsening of symptoms, the majority of patients experienced some adverse events (most commonly mild to moderate headaches), and up to 25% of patients experienced serious adverse events (Oudiz et al., 2012). Tadalafil appears to be a viable therapeutic option for patients unresponsive to sildenafil. However, further research is needed to evaluate the

long term efficacy and safety of tadalafil compared to sildenafil in chronic treatment plans.

Combination Therapy

Intravenous epoprostenol remains a frontline therapy for severe cases of PAH, but unfortunately, guidelines for physicians in terms of combination therapies are often convoluted. Different agencies may have similar but conflicting guidelines based on varying degrees of evidence. Many patients fail to respond, or progressively stop responding, to a single monotherapy and it has been postulated that a combined therapeutic treatment algorithm targeting several pathways may be needed for aggressive cases of PAH. Unfortunately, many patients show initial improvements to a certain drug but continually deteriorate over time; initial clinical trials are often not long enough to capture this decline, emphasizing the importance of long follow-up studies.

One study followed patients on bosentan monotherapy, and while they showed acute improvements in their physical capacities, the patients worsened below baseline values over a year (Hoeper et al., 2004). Adjunct therapy with sildenafil, while maintaining the same dosing of bosentan, proved effective in improving 6-minute walk distances during a follow-up period of 9 months (Hoeper et al., 2004). In a separate study examining combination therapy using bosentan, sildenafil, and inhaled iloprost, Hoeper and colleagues found a significantly improved 3-year survival rate of nearly 80% (Hoeper et al., 2005).

In contrast, combining treprostinil with either an ET-1 antagonist or a PDE-5i did not produce significant improvements to patient exercise capacity compared to either therapy alone (Tapson et al., 2012). In addition, roughly 22% of patients discontinued treatment due to adverse side effects (Tapson et al., 2012). These results underline the importance of studying the efficacy of combination therapies over the long term, as the added potential for adverse side effects may offset any modest therapeutic benefits. In fact, combination therapies involving guanylate cyclase enhancers and PDE-5 inhibitors, both acting through the NO pathway, is contraindicated in PAH patients due to the risk of fatal adverse effects (Enderby and Burger, 2015).

The value in combination treatment, particularly in advanced cases or unresponsive patients, cannot be overstated as it has the potential to save many lives. In some cases, the beneficial effects can be synergistic as opposed to simply additive. For example, activation of the ETA receptor by endothelin-1 may inhibit overall NO production by vascular endothelial cells (Barst, 2008). Thus, co-administration of a PDE-5i and sildenafil, an ETA antagonist, may act together to increase NO pathway action and synergistically increase efficacy. Other synergistic effects have also been found between sildenafil and inhaled iloprost (Ghofrani et al., 2002).

Currently, initial therapy for PAH patients in WHO functional classes 1 to 3 involve administration of CCBs after an acute vasoreactivity test (Galiè et al., 2013). Should patients fail the acute vasoreactivity test, or should CCB efficacy diminish due to tolerance, treatment recommendations depend on patient WHO functional class. In the most advanced cases (WHO functional class 4), intravenous epoprostenol therapy is

recommended (Galiè et al., 2013). In WHO functional class 2 or 3 patients, a variety of drugs may be started for initial monotherapy at the discretion of the prescriber. Should patients fail to adequately improve on monotherapy, physicians should begin sequential combination therapy while considering the possibility of lung transplantation (Galiè et al., 2013). Combination therapy may be more effective if therapeutics from different targeting pathways are added to the initial monotherapy, but close monitoring of side effects and drug interactions are needed. Interestingly, unless patients fall within the WHO functional class 4 category, no single front-line treatment recommendation is given (Galiè et al., 2013) and physicians must weight a variety of patient factors, such as route of administration and safety profile of various drugs, in the management of PAH.

Emerging and Future Treatments

Selexipag is an orally available IP prostacyclin receptor agonist which was approved by the FDA for PAH treatment in 2015 (Chakinala et al., 2017). It boasts an increased selectivity for the pulmonary vasculature over other prostacyclin medications due to its low affinity for non-IP prostanoid receptors (Asaki et al., 2015), minimizing systemic side effects. A phase 3 placebo-controlled clinical trial was able to demonstrate that treatment with selexipag slowed disease progression and reduced hospitalizations; however, it did not significantly affect mortality rate (Sitbon et al., 2015). Another study found that pulmonary arterial pressure could be reduced by nearly a third if selexipag was added in combination with drugs targeting other pathways (Simonneau et al., 2012). Selexipag represents a novel prostacyclin treatment for PAH patients, with promise of a

convenient dosing regime and lower risk for adverse side effects when compared to similar drugs in its class. Further research and pharmacovigilance is needed to assess the efficacy of chronic selexipag treatment.

Riociguat is an exciting and relatively new drug approved by the FDA in 2013; it is the first drug in its class to be approved for the treatment of WHO PH group 1 and 4 (Makowski et al., 2015). It acts as a soluble guanylate cyclase enhancer, increasing the levels of cGMP independently of the local availability of NO. This may provide an advantage over traditional PDE-5i therapy as PAH patients have lower levels of NO synthase (Ghosh et al., 2016), lowering overall NO production in the pulmonary vasculature. Phase 3 trials of riociguat have shown an increase in patient 6-minute walk distance of 36 m, as well as significant improvements to arterial resistance and pressure in the pulmonary vasculature (Ghofrani et al., 2013). A follow-up study also found that 6-minute walk distances continued to improve in patients for up to 24 weeks and significantly lengthened the time until patients suffered symptomatic worsening (Ghofrani et al., 2013). Although this novel therapeutic represents an exciting approach to PAH treatment, the clinical use of riociguat has several drawbacks. The drug has been shown to be teratogenic in animal models, and is therefore contraindicated in pregnant women (Makowski et al., 2015). Although rare, serious bleeding and hemorrhagic events may also occur, and the drug cannot be used in conjunction with PDE-5i therapy due to drug interactions (McLaughlin et al., 2015). Cost may also be a limiting factor for use, with prices nearing \$90,000 (Makowski et al., 2015).

Imatinib has been approved by the FDA as a chemotherapy agent for well over a decade; its effectiveness in PAH treatment is an emerging topic. Imatinib is a tyrosine kinase inhibitor currently used in the treatment of certain cancers, such as chronic myelogenous leukemia and acute lymphocytic leukemia. Its rationale for PAH treatment revolves around the vascular remodeling seen in the pulmonary circuit, particularly the disorganized proliferation of smooth muscle cells and fibroblasts seen in PAH (Humbert et al., 2004). Therefore, it is hypothesized that inhibition of growth factors, such as platelet-derived growth factor (PDGF), may reverse the effects of pulmonary vascular remodeling and provide an antiproliferative treatment for PAH. There have been several case reports showing efficacy of imatinib in PAH treatment, particularly in patients unresponsive to traditional therapies or with rapidly deteriorating conditions despite treatment (Ghofrani et al., 2005; Souza et al., 2006). Studies also showed that the drug could improve 6-minute walk distances by 36 m and modestly improve hemodynamics in patients already on combination therapy (Hoepfer et al., 2013). Further research is needed on the long term efficacy of using imatinib, particularly its effects on patient mortality, and as well as its safety profile in PAH patients.

Macitentan is a therapeutic directed against the ET-1 pathway approved by the FDA for PAH treatment in 2013. It is a dual ETA-ETB receptor antagonist taken once daily, and has a lower incidence of hepatotoxicity compared to other ET-1 antagonists (Raghu et al., 2013). Therapy was shown to significantly delay worsening of symptoms, and it also reduced death rates due to PAH by 13% when compared to a placebo group (Pulido et al., 2013). Patients also showed a modest improvement in 6-minute walk

distances and hemodynamic factors (Pulido et al., 2013); however, improvements were not as significant compared to results from other, more traditional therapies. While the therapy may show promise due to the lack of side effects normally associated with endothelin-1 antagonists, further research is needed on its value as an efficacious treatment option when used in combination.

Genetic Therapy

While great advancements have been made over the last two decades regarding targeted PAH therapy, there is still ample room to improve patient outcomes and mortality rates. Advancements in therapeutics, particularly those with reduced off-target effects and with an easier route of administration, have helped patients cope with pulmonary hypertension. Approaching treatment from a multifactorial view and addressing all three pathways implicated in pulmonary hypertension pathology may offer the best hope at slowing disease progression. It seems that no single pathway is entirely responsible for the disease progression in PAH, and dysfunction in more than one pathway may be the ultimate cause. Already, novel therapeutics and new research into the efficacy of combination therapies have shown to considerably improve patient quality of life, particularly in those patients unresponsive to traditional therapies. The rationale for recent advancements has largely been driven by the observation of an imbalance in endothelial-derived vasoconstrictors over vasodilators. Genetic therapy has the potential to address this pathobiology at its root, potentially reversing the effects of PAH on the pulmonary vascular structure. Since most patients present at the clinic once symptoms

worsen and the disease has progressed to a late stage, reversing the vascular changes caused by PAH may be much more efficacious than preventing further remodeling.

Although genetic therapy may offer the best hope of curing PAH in the future, current research in a clinical setting is limited. It is well known that the *BMPR2* gene is closely related to the occurrence of FPAH, and may be implicated in a minority of IPAH cases as well (Newman et al., 2004). In fact, up to 70% of FPAH cases and 10-40% of IPAH cases are correlated with mutations in the *BMPR2* gene (Sztrymf et al., 2008). To further complicate this matter, patients with *BMPR2* mutations may also respond poorly to vasoreactivity tests (Sztrymf et al., 2008), excluding them from conventional therapies such as oral CCBs. As such, genotyping of patients at risk for PAH for mutations in *BMPR2* may prove a useful diagnostic tool for future physicians. A definitive marker for poor prognosis in PAH patients is entering treatment at WHO functional class 3 and above; by identifying at-risk patients with the potential to develop PAH before widespread pulmonary vasculature remodeling has taken place, physicians may be able to drastically improve outcomes with the currently available therapies.

The importance of the *BMPR2* gene in hereditary PAH should be strongly emphasized as it represents a novel approach to treatment. Animal models were able to show that deletion of the *BMPR2* gene in endothelial cells was sufficient to induce symptoms of PAH (Hong et al., 2008). Protein replacement therapy and other genetic options are future pathways in which PAH research should be directed. A major ligand for *BMPR2* is the bone morphogenetic protein (BMP), of which there are many subtypes. Researchers were able to show that increasing the concentration of BMP can overcome

the loss of function related to mutations in *BMPR2* gene, in vitro (Yang et al., 2008). Thus, administration of a BMP ligand with high selectivity for the pulmonary endothelium may represent a novel approach to PAH treatment. Recent studies showed that administering BMP9 could not only reverse the effects of vascular remodeling found in mice with *BMPR2* mutations, but enhance *BMPR2* gene expression as well (Long et al., 2015). Future genetic research is needed to shed light on the complexity associated with this concept in human subjects.

Another gene which may be targeted for PAH treatment is survivin. Survivin is a member of the inhibitor of apoptosis (IAP) protein family and its increased expression is often associated with malignant cancers (Jaiswal et al., 2015). Interestingly, researchers found high levels of survivin in the pulmonary vasculature of patients with PAH (McMurtry et al., 2005). Furthermore, they were able to show survivin expression increases with increasing pulmonary arterial pressure. By suppressing endogenous survivin levels in the rat, researchers were able to reverse the adverse vascular remodeling and significantly improve survival rates (McMurtry et al., 2005). Its differential expression in the remodeled vasculature associated with PAH and relative absence in normal tissue makes survivin an appealing target for future therapies. There are a host of other potentially exciting areas of research in terms of genetic therapies for PAH. Researchers were able to show that injection of endothelial progenitor cells transfected with functional NO synthase could repair the vascular remodeling seen in PAH, and significantly reduce mortality rates in animal models (Zhao et al., 2005). The excitement surrounding cell-replacement therapies are indicative of their potential to

reverse the damage done by disease progression. Transfected endothelial progenitor cells were injected into PAH patients in a phase I clinical study: patients showed reduced arterial pressure, significantly improved physical capacity, and relatively low incidence of adverse effects (Granton et al., 2015).

Although genetic therapy as a treatment of PAH is in its infancy, advancements in research offer patients a glimpse towards a cure and the future of PAH management. To date, substantial advancements have been made in delivering targeted pharmaceuticals to patients, but most drugs act to prevent further deterioration of the pulmonary vasculature and do not reverse the effects of dysfunctional endothelial-driven arterial remodeling. A particular challenge in the clinical treatment of PAH is diagnosis: most patients do not present until the disease has progressed far enough to cause symptoms. Therefore, early diagnosis by biomarkers may be instrumental in improving patient outcomes with conventional therapies. With accelerated research and funds devoted to this disease, we may be able to significantly improve quality of life and prognosis for patients.

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CURRICULUM VITAE

