Pulmonary Arterial Hypertension: Challenges in Translational Research and a Vision for Change

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Pulmonary arterial hypertension (PAH) is a vascular remodeling disease with a relentless course toward heart failure and early death. Existing PAH therapies, all of which were developed originally to treat systemic vascular diseases, cannot reverse the disease or markedly improve survival and are expensive. Although there has been a recent increase in the number of potential new therapies emerging from animal studies, less than 3% of the active PAH clinical trials are examining such therapies. There are many potential explanations for the translational gap in this complex multifactorial disease. We discuss these challenges and propose solutions that range from including clinical endpoints in animal studies and improving the rigor of human trials to conducting mechanistic early-phase trials and randomized trials with innovative designs based on personalized medicine principles. Global, independent patient and tissue registries and enhanced communication among academics, industry, and regulatory authorities are needed. The diversity of the mechanisms and pathology of PAH calls for broad comprehensive theories that encompass emerging evidence for contributions of metabolism and inflammation to PAH to support more effective therapeutic target identification.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a complex vascular disease that results in heart failure and premature death. Although relatively rare, its impact on society is significant: PAH is a deadly disease that kills patients at a productive age and at a tremendous financial cost. The prevalence of PAH is estimated to be 15 to 50 per million, but this is likely an underestimation (1, 2). Within certain high-risk groups, the prevalence is higher: Among patients with HIV infection, the prevalence is 0.5% (3), and among patients with scleroderma, the prevalence is as high as 12% (4).

Although none of the currently approved therapies can reverse or cure PAH, the care and the quality of life of PAH patients have improved over the past 20 years, as described in recent reviews that summarize the progress in therapy and management of PAH (2), subjects only briefly discussed here. Nevertheless, an explosion of knowledge from preclinical models of PAH is being translated into humans at a frustratingly slow rate. We will discuss the many challenges for clinicians, pathologists, and scientists in the PAH field and will propose potential solutions to improve translation of basic discoveries to PAH treatment.

THE CLINICAL PROBLEM AND ITS IMPACT

PAH is a syndrome defined by an increase in the mean pulmonary artery pressure to more than 25 mmHg at rest with normal left ventricular filling pressures, in the absence of any secondary causes such as parenchymal or thromboembolic lung disease (2) (Table 1). PAH is characterized by decreased vascular perfusion (Fig. 1A), a result of proliferative remodeling in the medium- and small-sized pulmonary arteries that progressively decreases the cross-sectional area of the vascular lumen (Fig. 1B). The resulting rise in pulmonary vascular resistance stresses the right ventricle (RV), which, after short-lived compensatory hypertrophy (5), progresses to a decompensation phase and a decrease in cardiac output, progressive heart failure, and death. Because the pulmonary circulation has a higher capacitance and lower resistance stresses the right ventricle (RV), which, after short-lived compensatory hypertrophy (5), progresses to a decompensation phase and a decrease in cardiac output, progressive heart failure, and death. Because the pulmonary circulation has a higher capacitance and lower

Fig. 1. PAH is a complex vascular remodeling disease. (A) Magnetic resonance imaging (MRI) angiogram of the lungs in a healthy control individual (left panel) and a patient with PAH (right panel) showing enlarged pulmonary arteries and severely attenuated vascular perfusion of the distal pulmonary arteries in PAH. PA, pulmonary artery, RA, right atrium, modified with permission from (19). (B) Immunohistochemistry of distal pulmonary arteries shows a cross section from a healthy control (left panels), with a normal thin-walled vessel (arrow) around a wide lumen, surrounded by alveoli (hematoxylin and eosin (H&E) staining). Sections from a patient with iPAH (right panels) show, from left to right, intima thickening (H&E staining; nuclei, dark blue; cytoplasm, pink), media thickening (immunohistochemistry of smooth muscle cells; smooth muscle actin, brown), and a pleomorphic lesion (H&E staining) showing “anarchous” growth of cells that completely obliterates the vascular lumen. Scale bars, 100 μm.
5. Miscellaneous

5.1. Hematologic disorders: myeloproliferative disorders, splenectomy

5.2. Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis

5.3. Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders

5.4. Others: tumoral obstruction, fibrosing mediastinitis

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PAH can be idiopathic (iPAH) or heritable (hPAH) (6), or can be associated with other conditions (aPAH) such as scleroderma or other autoimmune collagen diseases, congenital heart disease, HIV infection, or anorexigen drug use (Table 1). The morbidity of PAH results from both the progressive vascular remodeling and the inability of the thin-walled RV to compensate adequately for higher pulmonary resistance (in comparison to the compensation of the left ventricle in response to systemic hypertension) (2). The exact survival rates in PAH are not known. Results from an unbiased, prospective 1980’s National Institutes of Health registry indicated 3- and 5-year survival rates of 48% and 34% (7), respectively, but these data predate current therapies and Doppler echocardiography, a major screening tool. More recent projections from smaller, country-specific registries suggest that the present 5-year survival is about 50%, and the largest U.S. database REVEAL indicates a 5-year survival of 57% (2, 8). This apparent improvement may be because patients are now diagnosed at an earlier stage. Subpopulations of aPAH patients (PAH associated with scleroderma or HIV infection) have 5-year survival rates of less than 40% or 20%, respectively (2).

Existing therapies cannot reverse the disease or significantly reduce mortality, although they improve patients’ quality of life and symptoms (9, 10). Three classes of medications are available: prostacyclin synthetics/analogs, endothelin receptor antagonists (ERAs), and phosphodiesterase type 5 inhibitors (PDE5i). Current practice specifies the use of an ERA or PDE5i as monotherapy in patients at earlier stages of the disease [World Health Organization (WHO) functional class II or III], with the use or addition of a parenteral prostacyclin synthetic/analog in advanced disease (WHO functional class IV) (2). Eventually, lung or lung/heart transplantation may be required for the many patients who continue to progress. The treatment of PAH is extremely expensive. For example, in the UK, the annual cost to treat a patient with advanced PAH with bosentan (an ERA) and epoprostenol (a synthetic prostacyclin) is $216,953 (current conversion rates) (11). In the United States, the annual cost of a patient treated with bosentan (an ERA) can reach $79,404 (12, 13). A lung/heart transplantation adds markedly to the cost of the disease, and the fact that women of reproductive age are the primary victims (2) magnifies its societal impact.

RECENT PROGRESS

Before the 1990s, we already had a basic understanding of pulmonary vascular biology [including the discovery of nitric oxide (NO), prostacyclin, and endothelin as major regulators]. Nevertheless, it was not until after the publication of seminal papers on human tissues and on patients with PAH that progression in treating PAH was made (Fig. 2). In 1993, the endothelin-1 axis was found to be up-regulated in lungs of patients with PAH (14), and in 1995, NO synthase was shown to be down-regulated in patients’ lungs (15). Along with previous animal studies, these results formed the basis of the “vasoconstrictor hypothesis” of PAH, which proposed that reversal of the apparent imbalance between vasoconstrictors (endothelin) and vasodilators (NO) in the pulmonary vasculature could be therapeutic. This was a welcome idea because there were no available therapies. A randomized trial showing that a vasodilator (intravenous epoprostenol) improved PAH (16) sparked an increase in publications, and about 10 years after the 1993 endothelin-1 paper, oral vasodilator therapy with the ERA bosentan was shown to improve PAH in a randomized trial (17). Both epoprostenol and bosentan were subsequently approved for use in the clinic, although the trials were short (3 months) and the efficacy was modest (an increase in the distance covered in a 6-min walk, the primary...
STATE OF THE ART REVIEW

Fig. 2. Number of publications on experimental PAH therapies after pivotal human studies. The number of publications addressing experimental therapies in PAH began increasing in 1995 after critical publications in human PAH tissues and PAH randomized clinical trials (see text and Supplementary Materials).

1. First observation of increased endothelin-1 expression in lungs of PAH patients
2. First observation of reduced endothelial NOS expression in lungs of PAH patients
3. First successful randomized clinical trial in PAH (intravenous epoprostenol)
4. First demonstration of BMPRII mutations in familial PAH
5. First successful randomized clinical trial in PAH with an oral agent (bosentan)

One outstanding hurdle to progress is that we lack a comprehensive theory(s) that explains the diverse features of PAH. The modest efficacy of the currently approved therapies for PAH may be a result of their targeting only one of several involved signaling pathways and fail to address the fundamentals of PAH pathology. However, to develop PAH-specific therapeutics, a unified understanding of the etiology and pathology of the disease is required.

Any comprehensive theory for PAH must explain the underlying features of this disease. These include an antiapoptotic and proproliferative diathesis, as well as a strong inflammatory diathesis that characterizes the remodeling vascular wall in medium and small pulmonary arteries. This diathesis is the response of the pulmonary vessels to diverse stimuli or disease triggers (hypoxia, germline loss-of-function mutations, infection with viruses like herpes simplex or HIV, etc.). An additional key feature of PAH is that, although many of the triggers are systemic, only the pulmonary vasculature is affected; systemic vessels are typically spared.

An ideal PAH therapy should inhibit proliferation and induce apoptosis (to reverse established lesions without the inflammatory consequence of nonapoptotic cell death) in the affected pulmonary vessels but not in systemic vessels. For example, a therapy that induces pulmonary vascular apoptosis nonspecifically may also cause aneurysms in systemic arteries. Also, an effective and pulmonary-selective therapy should not adversely affect the RV or, ideally, should improve its function.

Unique features of the pulmonary vascular system

The selective susceptibility of the pulmonary vasculature is a key feature that must be explained by any theory of PAH etiology. One distinction is its response to hypoxia. Because lungs are “distributors” of oxygen and the peripheral organs are “consumers” of oxygen, low endpoint, of 47 and 36 m, respectively). These successes have certainly enhanced the lives of PAH patients. Nevertheless, vasodilators are not as useful as initially hoped, because they do not treat the cause of the disease: PAH is not a result of vasoconstriction but rather a result of proliferative vascular remodeling (18, 19).

The vasodilator therapies, including the later-approved PDE5i, which activate the NO–soluble guanylyl cyclase (sGC)–cyclic guanosine monophosphate (cGMP) axis (20), were not developed primarily as PAH therapies. Epoprostenol and ERAs had failed in congestive heart failure and hypertension trials (because of worse patient outcomes) (21–23). Sildenafil (a PDE5i), initially formulated as a systemic vasodilator, was tested in hPAH when patents for its popular use in erectile dysfunction were due to expire.

In 2001, patients with hPAH were found to carry loss-of-function mutations in the bone morphogenetic protein receptor II (BMPRII) (24, 25). Signaling downstream of this receptor proved to be complex, and this work has not yet led to any definitive conclusions or promising therapeutic targets. Such mutations are found in most patients with hPAH (although these are rare) but in only a few patients with the more common sporadic (nonhereditary) PAH (2).

Over the past 15 years, a myriad of molecular factors have been shown to contribute to PAH in animals, and many have been effectively targeted in preclinical studies, as recently reviewed (18, 19, 26). These factors are diverse, ranging from potassium channels (27) to serotonin receptors (28, 29), vasoactive intestinal peptide (VIP) (30), survivin (31), Notch (32), tyrosine kinases (33), vascular elastases (34), mitochondrial metabolism (35–38), apelin (39), or transcription factors like nuclear factor of activated T cells (NFAT) (40), hypoxia-inducible factor 1α (HIF1α) (41), peroxisome proliferator–activated receptor γ (PPARγ) (42), or signal transducer and activator of transcription 3 (STAT3) (43, 44). This diverse array of targets makes it difficult to identify the most promising to translate to the bedside.

In the face of this plethora of possible drug targets, how are we performing in this critical stage of translational research? Of the 598 clinical trials for PAH registered at clinicaltrials.gov (Fig. 3), 393 explore PAH therapies. However, 328 of these test drugs are from one of the three already approved classes. Only 65 test new therapies, and of these, most are for drugs that are either generic or already used for other indications; none has been developed specifically for PAH. Overall, only 2.5% of all current trials test drugs that reverse the specific vascular pathology of PAH in preclinical studies. These include pyruvate dehydrogenase (PDH) kinase inhibitors (dichloroacetate), 5-hydroxytryptamine (5-HT) reuptake or receptor antagonists (PRX-08066, fluoxetine, and escitalopram), tyrosine kinase inhibitors (imatinib, sorafenib, and nicotinib), VIP, endothelial progenitor cell therapy, dehydroepiandrosterone (DHEA), PPARγ agonists (pioglitazone), CD20 antibodies (rituximab), and interleukin-1 receptor antagonists (anakinra) (Fig. 3). Most of these were developed primarily for other indications including depression, cancer, and rheumatologic or metabolic diseases. Why are there no drugs developed specifically for PAH, and why do so few trials investigate drugs that specifically address the current understanding of the pathology of PAH? The answers may lie in the challenges faced by clinicians, pathologists, scientists, and industry as they translate preclinical results into clinical applications.

WANTED: A UNIFYING THEORY OF PAH

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Fig. 3. Current clinical trials in PAH. There are 598 ongoing clinical trials on pulmonary hypertension (clinicaltrials.gov). Currently approved classes of drugs for PAH—prostacyclin synthetics/analogs, ERAs, and activators of the NO-cGMP axis [including phosphodiesterase 5 inhibitors (PDE5i) or soluble guanylyl cyclase (sGC) activators]—are being tested in 328 of these clinical trials. Two hundred five PAH clinical trials investigate clinical outcomes in cohorts or registries of PAH patients (survival, quality of life, and number of hospitalizations), biomarkers, and supportive therapies, whereas only 65 clinical trials test new PAH therapies. Of these 65 trials, which are based on strong preclinical evidence of reversal of the proliferative vascular remodeling, 21 are funded by industry and 44 by other funding sources. Most of these therapies have been developed to treat other common diseases, not PAH, such as depression, rheumatologic diseases, or cancer. ERAs, endothelin receptor antagonists; NO, nitric oxide; GC, guanylyl cyclase; DCA, dichloroacetate; EPC, endothelial precursor cells; SMC, smooth muscle cells; APJ, apelin receptor.

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<tr>
<th>Drug intervention</th>
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<th>Clinical trial sponsor and collaborator</th>
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<tr>
<td>PRX-08066</td>
<td>Serotonin receptor antagonist</td>
<td>Epix Pharmaceuticals</td>
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<td>EPC transplantation</td>
<td>Stem cell therapy</td>
<td>Northern Therapeutics, St. Michael’s Hospital, Sir Mortimer B. Davis (Jewish General Hospital)</td>
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<td>Tyrosine kinase inhibitor</td>
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<td>Ranolazine</td>
<td>Fatty acid oxidation inhibitor</td>
<td>Northwestern University, Gilead</td>
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<td>DCA</td>
<td>PDK inhibitor</td>
<td>University of Alberta, Imperial College</td>
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<td>Pioglitzone</td>
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<td>VIP</td>
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<td>Ribonuclease reductase inhibitor</td>
<td>Children’s Memorial Hospital</td>
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oxygen levels cause the pulmonary arteries to constrict and the systemic arteries to dilate. This hypoxic pulmonary vasoconstriction (HPV), intrinsic only to the lungs, is present in all mammals and maintains ventilation-perfusion match, because constriction of a pulmonary artery perfusing a hypoxic area redirects the blood to better-oxygenated areas of the lung (45). The mitochondria of pulmonary arterial smooth muscle cells (PASMCs), which serve as oxygen sensors in many tissues, are the basis of this pulmonary response (45). In hypoxia, the mitochondria alter the production of mitochondria-derived reactive O2 species (mROS), which diffuse to regulate redox-sensitive targets. These targets, such as the plasma membrane voltage-gated potassium channels (Kv) or redox-sensitive components of the HIF1α signaling pathway, induce acute PASMC contraction (via the intracellular calcium increase that follows Kv channel inhibition) or initiate the response to a more sustained hypoxia by the activation of the many HIF1α-regulated genes. PASMC mitochondria are quite distinct from mitochondria in systemic arteries (for example, they have altered expression of complex I proteins and manganese superoxide dismutase), potentially explaining the restriction of HPV to the pulmonary circulation (46).

Mitochondria are also critical regulators of apoptosis, can induce proliferative and inflammatory signals, and at the same time can integrate other molecular signals such as oncogenes, growth factors, and endoplasmic reticulum (ER) stress (47). These unique features of mitochondria may contribute to PAH and could therefore be targets for selective and effective proapoptotic and antiproliferative therapies for PAH (48).

The contributions of metabolic dysfunction and inflammation to PAH
Mitochondrial function, specifically glucose oxidation, is suppressed in both PAH PASMCs and endothelial cells (37, 38, 49). The consequences of this suppression are multiple.

(i) The suppression of glucose oxidation and a metabolic switch toward glycolysis is associated with a state of resistance to apoptosis via several mechanisms, reviewed elsewhere (50). Mitochondria become hyperpolarized and mROS decreases (37, 38, 51–54). This results in an increase in the opening threshold of the mitochondria transition pore, a mega channel through which proapoptotic factors (like cytochrome c or apoptosis-inducing factor) move to the cytoplasm (55). The inefficiency of adenosine triphosphate (ATP) production that follows the suppression of mitochondrial function results in a secondary up-regulation of glucose uptake and glycolysis to generate ATP in the cytoplasm. This is in keeping with higher uptake of a radiolabeled glucose analog in the lungs of PAH patients than in those of healthy controls (Fig. 4A) (49). Many up-regulated glycolytic enzymes also have secondary antiapoptotic properties (50).

(ii) The levels of mROS and diffusible mitochondria-derived mediators like α-ketoglutarate (αKG) decrease. This has multiple downstream effects. For example, plasma membrane redox-sensitive Kv channels are inhibited, leading to a secondary influx of calcium to the cell (45, 56). Many pro-proliferative, calcium-sensitivity transcription factors are activated, including NFAT. Also suppressed is NFAT’s inhibitor, the metabolic enzyme glycogen synthase kinase 3β (GSK3β), a direct result of the metabolic switch toward glycolysis (37, 57). NFAT not only activates proliferative signals but also down-regulates Kv channels and promotes global mitochondrial suppression, closing a positive feedback loop (58). NFAT is critical for PAH, and its inhibition can reverse PAH in animal models (40).

The decrease in mROS and αKG also promotes activation of HIF1α, another master transcription factor that is activated in animal and human PAH, even in the absence of hypoxia (41). HIF1α can be activated directly by suppressed mitochondrial signaling via both redox-sensitive and redox-insensitive mechanisms (54). Like NFAT, HIF1α also suppresses Kv channels and directly promotes proliferation (41).

(iii) Although yet unexplored in PAH, mitochondrial suppression may also activate the inflammasome (59), which could cause a cascade of events that increases autocrine or paracrine cytokines (60) and recruits inflammatory cells to the diseased pulmonary circulation (61).

Thus, mitochondrial suppression could explain diverse, apparently unrelated, features of the PAH phenotype: hyperpolarized mitochondria, activated NFAT and HIF1α, inhibition of Kv channels, increased cytosolic calcium levels, and activation of inflammation. If this suppression is global and includes, for example, peripheral muscles, it could also explain the insulin resistance (not related to obesity or diabetes) recently described in PAH patients (62). Indeed, suppressed oxidative phosphorylation in skeletal muscle mitochondria contributes to the metabolic syndrome (63, 64).

What is the proximal cause (or upstream mediators) of this mitochondrial suppression in PAH? One candidate is the gate-keeping enzyme for glucose oxidation, mitochondrial PDH, which is inhibited in several PAH models (35–38, 53). This suppression may be caused by upstream regulation by its inhibitor PDH kinase (PDK), a HIF1α-inducible and a tyrosine kinase receptor–activated enzyme (65, 66), and an important therapeutic target (35–37). Another regulator of PDH is mitochondrial calcium, which, when suppressed, decreases both PDH and general mitochondrial function. The ER stress that accompanies many of the triggers of PAH (infections, BmprII mutations, and toxins) (3, 67–71) would decrease calcium entry from the ER as part of stress-induced anatomic remodeling of the ER-mitochondria unit (38). There are also fewer mitochondria in PAH tissues, a result of abnormalities in the master regulator PGC1α or in proteins like mitofusin-2 that regulate mitochondria networking during biogenesis (72). Genetic polymorphisms in mitochondrial regulators such as Sirt3 and Ucp2 have also been linked to metabolic and vascular diseases in humans (73, 74). Intriguingly, the absence of Ucp2 (uncoupling protein 2; a mitochondrial protein that regulates calcium entry into the mitochondria) causes mitochondrial suppression in pulmonary artery smooth muscle cells. Ucp2-deficient mice, which have features of insulin resistance, develop spontaneous pulmonary hypertension, associated with activation of NFAT and HIF1α, even under normoxia (75).

Normalization of these causes of mitochondrial suppression can reverse PAH in animal models. Activation of PDH, directly (with the small-molecule PDK inhibitor dichloroacetate) (35, 37, 76) or indirectly (with activators of fatty acid oxidation) (37), mitofusin-2, or PGC1α (72) or inhibition of ER stress with phenylbutyrate or salubrinal (61, 77) normalizes both the cellular and the in vivo phenotype of the disease, including seemingly unrelated abnormalities such as NFAT or HIF1α activation or inhibition of Kv channels, promoting apoptosis and reversal of the PAH vascular remodeling selectively in the pulmonary circulation.

Another overarching hypothesis for PAH that can potentially explain several PAH-related abnormalities is inflammation, as discussed in a recent review (60). Sterile inflammation characterizes animal pulmonary hypertension models (like the hypoxia and monocrotaline models) and is an increasingly recognized feature of clinical PAH (78–80). In addition, PAH patients with generalized inflammation (for example, connective tissue diseases and HIV) have worse prognosis...
and clinical outcomes than those without inflammation (81). Immunosuppressive therapy causes improvement in a subset of PAH patients, supporting the relevance of inflammation to PAH (82). Remodeled vascular walls and the perivascular region of PAH lungs contain inflammatory infiltrates (83): T cells (84), B cells (85), macrophages (86), neutrophils (86), and mast cells (87) (Fig. 4B). Inflammatory cytokines such as interleukins, stromal-derived factor–1, monocyte chemoattractant protein–1, and tumor necrosis factor–α (TNFα) are found in the serum of PAH patients (88, 89), often correlating with disease severity and prognosis (88). Targeting these immune cells or cytokines [like T helper 2 (Th2) cells, interleukin-1, and TNFα] can reverse PAH in animals (53, 90, 91).

Because suppressed mitochondria can induce the inflammasome (92), both metabolic defects and inflammation may contribute to the pathogenesis of PAH (Fig. 4C). Many of the inflammatory mediators elevated in PAH can also suppress mitochondrial function. For example, TNFα can inhibit PDH in PASMCs and induce the PAH cellular phenotype in normal PASMCs (53). Inhibition of TNFα with etanercept normalizes the metabolic phenotype in animal PAH and reverses the disease in vivo (53). On the other hand, activated immune cells show suppressed mitochondrial function and develop a glycolytic phenotype, similar to PAH vascular cells (93, 94). As in PAH PASMCs in animal models, the circulating immune cells of PAH patients show ER stress (95), a state that is associated with increased synthesis of cytokines (95). These findings suggest that there is a mutually reinforcing interplay between the vascular cells and activated immune cells that amplify and sustain the PAH phenotype in vivo.

Patients with the familial disease Dursun syndrome exhibit iPAH, abnormal white blood cells, and atrial septal defects in children; these patients carry a causal homozygous mutation in G6PC3, the gene encoding the glucose metabolism enzyme glucose-6-phosphatase (96, 97). The abnormalities in inflammatory cells and glucose homeostasis in these patients may be related to their PAH, mimicking the suppressed glucose oxidation and activated inflammation in both humans and animals with PAH. The characteristics of PAH seen in patients with Dursun syndrome support an underlying combined metabolic and inflammatory deficit in this disease.

Fig. 4. An emerging comprehensive theory for PAH: metabolism and inflammation. (A) Metabolic alterations in PAH. Four iPAH patients show higher glucose uptake in the lungs than do three healthy controls, as assessed by 18-fluorodeoxyglucose uptake (18-FDG), suggesting a cancer-like switch in metabolism toward glycolysis. The lung standardized uptake values (SUV) were normalized to lung tissue fraction. Mean data are shown on the right (*P < 0.01, multiple comparison test). Modified with permission from (49). (B) Inflammation in PAH. Cross sections of pulmonary arteries from PAH lung are remodeled, with dense inflammatory cell infiltrates (left panel, black arrow) or with concentric intima thickening (right panel, red arrow) surrounded by a dense infiltration of inflammatory cells (right panel, black arrow) (Russel-Movat pentachrome stain; nuclei, black; smooth muscle, red; mucin, blue). Modified with permission from (80). Scale bar, 100 μm. (C) Diagram showing ER stress and mitochondrial dysfunction in PAH. Suppressed mitochondrial function in smooth muscle cells of PAH pulmonary arteries causes increased intracellular calcium (Ca2+) and decreased generation of mitochondrial-derived reactive oxygen species (mROS), resulting in activation of pro-proliferative transcription factors like NFAT and HIF1α. Suppressed mitochondrial function can result through one of two ways: (i) ER stress (which causes a disruption of the ER-mitochondrial unit and decreased mitochondrial calcium, in turn inhibiting many calcium-sensitive mitochondrial enzymes such as pyruvate dehydrogenase), or (ii) suppression of pyruvate dehydrogenase by inflammatory cytokines such as TNFα, resulting in apoptosis resistance. ER stress can also increase the production of cytokines by inducing the unfolded protein response (UPR), resulting in the recruitment and activation of inflammatory cells. Inflammatory cells (characterized by a similar suppression of mitochondrial function) are activated and recruited to the lungs of PAH patients and can increase the cytokines that induce ER stress or directly suppress mitochondrial function in PAH smooth muscle cells of PAH pulmonary arteries. Cytokine production by PAH inflammatory cells can also have autocrine effects on mitochondrial function. PAH immune cells also show ER stress, which activates NFAT and upregulates many cytokines. This interplay of vascular and immune/inflammatory cells in PAH further reinforces and sustains the molecular phenotype of the disease.
TRANSLATIONAL CHALLENGES FOR THE PATHOLOGIST

The pathology of PAH has not been systematically described

Obliterative intima and media thickening, consisting mainly of the proliferation of smooth muscle cells and myofibroblasts, as well as of plexiform lesions (disorganized growth of proliferative endothelial cells), form the foundation of PAH pathology (Fig. 1B). PAH pathology was originally described in one cohort by Heath and Edwards in 1958 (98), and since then in 10 other cohorts, a total of 609 cases (80). Although the heterogeneity of the vascular lesions within a lung of individual patients has been recognized, the sampling strategy has not been standardized and consistent. Additionally, there has been no systematic attempt to make clinical-pathological correlations, a particular problem because the definition of PAH has been expanding over the past 20 years. Many pathologists still use the Heath-Edwards grading of vascular lesions from 1958, which was based on a cohort of 67 lungs with PAH secondary to congenital heart disease and only 2 lungs from patients with iPAH. This classification described worsening vascular pathology, from grade 1 (medial hypertrophy) and grade 2 (intimal hypertrophy) to grade 5 (plexogenic lesions), giving the (untested) impression that this describes the natural history of disease progression. This oversimplification, along with scarce tissue for study, forced the field away from the clinicopathological approach to the disease, one of the cornerstones of medicine.

To address several of these limitations, in 2006, the Pulmonary Hypertension Breakthrough Initiative established a network of 13 multidisciplinary research and transplant centers. Whole lungs from transplant surgery (rather than lung samples or biopsies), cells, and blood were retrieved along with relevant validated clinical data. This systematic analysis of 62 PAH and 28 control lungs (80) showed that plexogenic lesions were found in most but not all PAH samples, whereas media and intima thickening were found in all. On several occasions, some slides from the same patient showed no plexogenic lesions, whereas others showed as many as 27 lesions. This confirmed the notion that the pathology of PAH is heterogeneous. Media thickening, but not plexogenic arteriopathy, correlated with mean pulmonary artery pressure and pulmonary vascular resistance. No differences in any of the lesions were found with current PAH therapies, with the exception of prostacyclin and its analogs, which correlated with the number of plexiform lesions. In some patients, plexiform lesions were present in the absence of media or intima thickening, challenging the notion that the plexiform lesions are the most advanced pathologic finding in PAH in a disease progression continuum. Many of the control lungs also showed media and intima thickening, supporting the need for larger sample sizes and biobanks of PAH tissues from patients with reliable and detailed clinical data.

Another important finding from this study (80) was the unexpectedly strong perivascular and interstitial inflammation (infiltration with lymphocytes, macrophages, and neutrophils) in all PAH lungs. Although the sample size was relatively small, specific patterns did emerge: Muscular remodeling (media and intima thickening) plus plexiform lesions showed 94% concordance with the clinical diagnosis of iPAH. In contrast, interstitial matrix remodeling, muscularization, inflammation, and fibrosis showed 42% concordance with the clinical diagnosis of iPAH. Different molecular phenotypes may underlie these pathology patterns, undermining the idea that a therapy showing efficacy in one type of PAH will be beneficial in another. The use of more than one animal model in preclinical research may be necessary to cover the spectrum of disease pathology.

Response: Systematically collect and analyze human PAH tissues. Expansion of the methodology of the Pulmonary Hypertension Breakthrough network, including methods to preserve macromolecules for mechanistic correlation studies, should be a priority for the field. Broader national and international networks for tissue harvesting and processing need to be developed and synchronized to establish large tissue banks accessible to all investigators. At this point, the harvesting of PAH tissues is limited to transplant surgery centers, although catheters for percutaneous endovascular biopsies to enable longitudinal studies in living PAH patients are under development (99).

Response: Understand the natural history of disease progression. The earliest lesion(s) in PAH and its triggers are unknown. The hypothesis that the earliest lesion is endothelial cell injury, which leads to pulmonary arterial endothelial cell apoptosis, followed by pathological repair and regeneration of the vasculature and recruitment of proliferative precursor cells, is intriguing but unproven (85). PAH tissues collected at the time of transplant will not elucidate this question because the disease is then at a highly advanced stage. Molecular imaging approaches that could detect apoptotic endothelial cells (similar to what is being developed in oncology) may prove to be useful (100).

TRANSLATIONAL CHALLENGES FOR THE CLINICIAN

All types of PAH are not likely to be equivalent

Several international meetings have proposed a classification scheme for PAH that groups conditions of diverse origin under the umbrella of PAH (6) on the basis of the fact that the lung vascular pathology is presumed to be similar (Table 1). After the pivotal clinical trial of bosentan (17), which primarily included patients with iPAH, the Food and Drug Administration approved the drug for several aPAH conditions not included in the trial. This presumed similarity between iPAH and aPAH has not been systematically verified, however, and it is not clear that the various types of PAH share the same pathology and that treatment should be the same among them. For example, hPAH caused by BMPRII loss-of-function mutations (which dysregulate SMAD signaling downstream of the BMPRII receptor) may be distinct from anorexigen-induced PAH (in which serotonin homeostasis and voltage-gated potassium channels are dysregulated in pulmonary vascular cells) or forms of PAH with activated inflammatory signaling (such as scleroderma-associated PAH). Indeed, as discussed above, systematic analyses of human PAH lung samples are revealing distinct phenotypes. Our present classification of PAH may not be adequate to predict whether a patient with a specific form of PAH will benefit from a treatment that has shown benefit (or not) in another form of PAH.

Response: Reexamine the clinical classification of PAH. The recent progress in the molecular understanding of the disease and recent reassessments of PAH pathology should be taken into account. A “metabolic” or an “inflammatory” phenotype, for example, may be more relevant for management and choice of therapy than the current classification of PAH.

RV function as a key determinant of PAH outcome is not reflected in clinical practice or in research

The most important determinant of morbidity and mortality in PAH is not the severity of the vascular pathology, as previously assumed,
but rather the function of the RV (2, 5), a poorly understood aspect of PAH. The characteristics of the left ventricle cannot be extrapolated to the RV because the two chambers have distinct embryology and differences in metabolism and physiology (101). The response of the RV to pressure overload is very different, and the compensation phase (the phase in which RV remodeling prevents a decrease in cardiac output) is much shorter than that of the left ventricle. In some PAH patients, pulmonary vascular resistance decreases in response to therapies (suggesting a positive response to treatment), but the RVs continue to deteriorate and the disease progresses clinically (102). Indeed, MRI indices of RV dysfunction predict clinical worsening in patients with PAH, independent of pulmonary artery pressure (103). These data challenge the generally accepted idea that RV failure is a linear and predictable consequence of progressive vascular remodeling. RV-intrinsic molecular mechanisms may drive primary RV deterioration, independent of the pulmonary vascular changes (104).

Studies of PAH may not directly assess RV function. Many approved PAH therapies have failed in previous trials of congestive heart failure, but their effects on the RV were never studied before clinical development for PAH. Although it is now becoming clear that it is the RV function and its response to therapy that determine morbidity and development for PAH. Even though the 6-min walk test was chosen for the first randomized trial in PAH (epoprostenol) (suggesting a positive response to treatment), the RVs continue to deteriorate and the disease progresses clinically (102). Indeed, MRI indices of RV dysfunction predict clinical worsening in patients with PAH, independent of pulmonary artery pressure (103). These data challenge the generally accepted idea that RV failure is a linear and predictable consequence of progressive vascular remodeling. RV-intrinsic molecular mechanisms may drive primary RV deterioration, independent of the pulmonary vascular changes (104).

Response: Study the RV and pulmonary circulation as a unit. It is not enough to show that an experimental therapy improves the pulmonary vascular remodeling or decreases the mean pulmonary artery pressure. Without improvement in RV function and the cardiac output, there will be no net benefit for the animal or the patient. A therapy that suppresses RV function can appear to cause a decrease in pulmonary artery pressure, but in reality, the response is a result of RV pump failure not of improvement in pulmonary vascular remodeling. Thus, the direct effects of any promising therapy for PAH should be tested on RV myocardium in vivo and ex vivo to make sure that there is a net improvement in cardiac output in vivo as well as in functional capacity and survival (106). For example, PDE5i and ERAs both decrease pulmonary artery pressure, but they have opposite effects on the RV when the RV is studied ex vivo: PDE5i increase (107) whereas ERAs decrease (108) RV myocardial inotropy (Fig. 5, A and B), perhaps explaining why edema (a potential result of suppressed RV contractility) is much more common in patients treated with ERAs.

Current drugs do not improve patient survival or hemodynamic function and clinical trials do not use strong and mechanistic endpoints

Current clinical guidelines (2) for PAH are based on trials in which the primary endpoint is the distance that a patient can walk in 6 min. This test was chosen for the first randomized trial in PAH (epoprostenol) to reflect the patient’s symptoms and functional performance (16). All later trials used the same endpoint, and all current PAH therapies were subsequently approved on the basis of the prolongation of the 6-min walk by about 40 m. Although statistically significant, this increase is not clinically meaningful or predictive of outcomes, including survival, hospitalizations, or need for heart-lung transplantation (109, 110). A supervised 10-week exercise protocol without any therapy improves quality of life indices and increased the 6-min walk more than 40 m in PAH patients (111).

Reliance on drugs that only improve the 6-min walk time but not more meaningful endpoints can give the false impression that there are many good therapy options available to PAH patients. This situation hinders translational efforts of new and more effective therapies.

Response: Develop drugs with new modes of action, relevant to PAH-specific pathology. Most drugs being tested clinically in PAH (outside of the three approved classes of drugs) are either generic or approved for other, non-PAH–related diagnoses. This may be indicative of the barriers to investment in the clinical development of drugs for rare diseases such as PAH, although the Orphan Drug Act can offer assistance (112). Creative approaches to intellectual property law will be important.

Response: Identify and validate biomarkers for PAH. Efforts need to be made to develop therapy-specific biomarkers, particularly using molecular imaging approaches. For example, a metabolism-based imaging biomarker (like glucose uptake studies with positron emission tomography) will be important for the assessment of metabolic modulators. An imaging biomarker that can detect apoptosis in vivo could be critical for the assessment of a proapoptotic therapy in vivo (100).

Response: Confirm preclinical animal results in mechanistic early-phase human trials. Currently, early-phase trials focus on safety/toxicity and potential efficacy assessed on the basis of nonmechanistic endpoints such as tolerance, toxicity, 6-min walk, and pulmonary artery pressure. One aim of such trials should be to identify the molecular phenotypes of patients most likely to respond to a particular therapy, with the goal of validation of therapy- and disease-specific biomarkers. Early-phase trialists (clinician scientists that often also perform preclinical work) should be embedded in networks of preclinical research to facilitate efficient translation. In addition, such networks can develop and support much-needed industry-independent blood and tissue registries. Trial designs with adaptive protocols and statistical approaches for rare diseases are being developed and should be considered for PAH trials (113–115).

Response: Identify specific subgroups of patients. Defined molecular phenotypes may allow later-phase trials in specific subgroups of patients with higher chance for positive response. For example, patients who show evidence of decreased PDH activity (measured in vivo by either hyperpolarized carbon-13 MRI (116) or by a breath test of 13C-labeled pyruvate (117)) may be the best candidates for a drug that increases PDH activity (such as the small-molecule dichloroacetate). Patients with a generalized metabolic abnormality (for example, insulin resistance based on standard blood tests) may be better candidates for a systemically delivered metabolic modulator; patients with BMPRII mutations may be the best candidates for a therapy that enhances downstream BMPRII signaling.

Response: Late-phase clinical trials should seek reversal of disease. The objective of clinical trials should be the true reversal of disease with minimal toxicity in prespecified patient subgroups with specific molecular phenotypes (118, 119). Protocols need to be extended to at least a year so that survival, sustainability of the effect, and long-term toxicity can be explored in a meaningful manner (118).

Response: Base clinical practice on trials with strong endpoints. Future treatment guidelines should be based on clinical trials in which efficacy is supported by strong endpoints such as survival or improvement in hemodynamics (pulmonary vascular resistance measured invasively or RV function).
Another model, which is being increasingly used, better mimics human PAH histology (severe intima thickening and severe obliterative vascular lesions) but requires an injection of a vascular endothelial growth factor (VEGF) inhibitor (the Sugen compound) in tandem with hypoxia (122). However, in PAH, VEGF is up-regulated in the remodeled pulmonary vessels (123), and kinase inhibitors that inhibit VEGF are being tested as potential therapies for clinical PAH (124, 125). It is therefore not clear whether VEGF inhibitors promote or inhibit PAH, creating confusion when this model is used for therapeutic development with such drugs.

One of the few animal models that develop spontaneous PAH is the fawn-hooded (FH) rat in which spontaneous PAH is accelerated by chronic hypoxia (41, 126). The precise molecular mechanism of PAH in these animals is unknown. A small subset (<5%) of S100A4/Mts1-overexpressing mice, which develop metastatic mouse mammary adenocarcinoma, also develop plexogenic-like lesions similar to human PAH (127), but the phenotype is very rare, making use of these mice to study PAH impractical. Although one study suggested that BmprII-/- mice developed moderately elevated mean pulmonary artery pressures (128), others using the same mice showed no significant changes (129, 130). In addition, mice with constitutively decreased BmprII expression did not exhibit changes in pulmonary vascular resistance (131). In contrast, a mouse strain with forced overexpression of a human BmprII mutation developed significant PAH (132), although the forced overexpression likely causes ER stress, confounding the underlying mechanism (humans do not overexpress the mutated protein).

There is no perfect animal model of human PAH, although several aspects of human PAH can be recreated (121). The lack of plexogenic arteriopathy in the MCT, chronic hypoxia, or FH rat models may not be a fatal weakness because the plexogenic lesion may not be the sine qua non of human PAH.

**Response: Use multiple animal models for preclinical research.**

The increasingly apparent heterogeneity of PAH pathology necessitates the use of different models because none reproduces all of the aspects of PAH pathology. At least two animal models and human tissues should be used in each preclinical study. It is also important to prove that a therapy works through the same mechanism in rodents as in human vascular cells (ideally derived from patients with PAH or healthy tissues).

**Clinically relevant endpoints are rarely used in preclinical PAH studies**

Most preclinical investigations of PAH examine the response of rodents to an experimental therapy after 3 to 4 weeks of treatment. This...
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period is not adequate to determine whether the effects are sustained or temporary, to detect long-term toxicity, or to explore effects on survival. Nevertheless, assessment of these endpoints is essential for informative preclinical studies, as is exploration of bone marrow and liver toxicities, to avoid the emergence of these problems later. Furthermore, the functional capacity of the treated animals is not typically measured (for example, with rodent treadmill tests). Cardiac output is often not reported. An additional weakness of many preclinical studies is the measurement of RV systolic pressure instead of mean pulmonary artery pressure, a result of the fact that it is much easier to insert a high-fidelity catheter in the RV than in the main pulmonary artery. Although the RV systolic pressure is usually the same as the systolic pulmonary artery pressure, this method does not measure true mean pulmonary artery pressure, precluding accurate calculation of pulmonary vascular resistance [(mean pulmonary artery pressure – pulmonary artery wedge pressure)/cardiac output], an essential endpoint in both animal and clinical studies (133). Promising PAH therapies should cause a measured decrease in pulmonary, but not systemic, vascular resistance. Possible direct effects of potential therapies on the RV are also rarely assessed, despite the recognized importance of RV function in the overall prognosis of PAH (134). In summary, preclinical animal studies do not focus on endpoints that are clinically relevant, including exercise capacity, survival, pulmonary vascular resistance, RV function, and toxicity.

Response: Use clinically relevant endpoints in animal research.

For optimal translation, preclinical studies should incorporate comprehensive hemodynamic assessment, functional capacity, long-term studies for toxicity, and survival assessment. Only then can an experimental therapy be properly assessed for potential translation to human studies, increasing the confidence of trialists and sponsors in eventual clinical success.

THE FUTURE

Preclinical progress in PAH research can be accelerated with a transparent and robust multicenter approach. Networks of centers with expertise in PAH preclinical research should be assembled in a manner similar to the network established for the collection and study of transplant material from PAH patients. Such networks could prioritize research questions and aggregate data and allow for network-wide monitoring and adherence to good preclinical research principles (record keeping, quality of data, reproducibility among different centers, etc.). Results on a potential therapy confirmed in several models and with much higher sample sizes than are typically seen in single laboratory studies will be more robust and reliable, addressing potential investigator-based scientific biases (Table 2).

Implementation of the changes recommended above would also be facilitated with the guidance of a clinical trial networks similar to the Thrombolysis In Myocardial Infarction groups for myocardial infarction trials (135) or the National Cancer Institute clinical trials networks (136, 137). Trials conducted with the guidance of such networks have other advantages, including the ownership and transparency of the trial data. Currently, data are available only to the sponsor company, particularly if the results are not positive. Such an organization would allow synergistic partnerships with industry but would also facilitate the trials with generic/orphan drugs that are increasingly promising for diseases such as PAH (Fig. 3). Increased communica-

Table 2. Translational priorities for the future of PAH research.

<table>
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<td>2. Comprehensive approach to the RV-PA unit</td>
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<td>8. Early-phase mechanistic studies following personalized medicine principles</td>
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<td>4. Pursuit of comprehensive theories for PAH</td>
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<td>5. Networks of excellence in preclinical PAH research</td>
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tion among the stakeholders, including scientists, trialists, industry, and regulatory authorities will be catalytic in allowing the investment for trials testing such compounds.

Efforts to increase the communication among all PAH stakeholders have begun. For example, the Pulmonary Vascular Research Institute (PVRI) (an international nonprofit network of scientists and clinicians with a mission to promote research and awareness of PAH globally) has started hosting meetings between PAH experts, industries, and regulatory authorities from the United States, Canada, and the European Union. The development of a common language among all stakeholders will be important for the future.

Most cases of PAH occur in developing countries, where randomized trials are more difficult to conduct and access to therapies is impossible. For example, one of the most common causes of PAH globally is schistosomiasis, which is almost nonexistent in the developed countries, and thus, it is rarely discussed or considered in clinical trials (138). PAH associated with HIV or with uncorrected congenital heart disease is also much more prevalent in developing than developed countries (138). One of the goals of PVRI is to establish an international, unbiased, and independent clinical database to understand the true incidence and prevalence of the disease globally, including in developing countries (139). This will both facilitate the design of truly global trials and increase the potential users of the developed therapies, benefiting industry.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/5/208/208sr5/DC1

Methods

REFERENCES AND NOTES

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