Pulmonary arterial hypertension (PAH) is a progressive disease of the lung vascular system, primarily affecting small pulmonary arterioles. A combination of endothelial dysfunction and increased contractility of small pulmonary arteries (PAs), proliferation and remodeling of endothelial and smooth muscle cells, and in situ thrombosis leads to progressive narrowing of the blood vessels. This results in a progressive resistance to blood flow and an increase in PA pressures. According to the clinical classification of pulmonary hypertension (PH; see Figure 1) from the Fifth World Symposium (Nice, 2013). PAH can be idiopathic (IPAH, caused by reasons that are unknown), heritable/familial, or associated with other medical conditions such as connective tissue disease, HIV infection, portal hypertension (liver disease), sickle cell disease, and congenital heart disease. PAH has also been associated with drug and toxin exposures, especially to the use of anorexigens, such as fenfluramine and dexfenfluramine as well as toxic rapeseed oil. Rarely is PAH caused by pulmonary veno-occlusive disease or is related to persistent PH of the newborn.

There are other pathologies in which PH presents as a secondary disease, and it is always important to rule out secondary disease during an initial comprehensive evaluation of a patient with PAH. Some examples are shown in Figure 1 and include left heart disease (group 2), chronic lung diseases and hypoxia (group 3), chronic thromboembolic PH (group 4),
or PH related to other unclear/multifactorial mechanisms (group 5, eg, lymphangioleiomyomatosis, histiocytosis, and sarcoidosis). It is important to note that the diagnosis of PAH, particularly IPAH, is a diagnosis of exclusion.

The evaluation of a patient with suspected PAH requires a combination of tests, including history, physical examination, echocardiography, imaging, laboratory screenings, functional assessment/exercise tolerance, and right heart catheterization. For a standard approach to the diagnosis of PAH at our institution, see Figure 2. Classic symptoms of PAH, such as fatigue, lethargy, dyspnea, and exertional syncope/syncope, are often the first clue to PH. Physical examination findings will depend on the severity of the disorder in the pulmonary vasculature and the degree of right ventricular dysfunction. In all subtypes of PH, a loud P2 (second pulmonic closure sound) is frequently heard in association with tricuspid regurgitation murmur. A right-sided third or fourth heart sound may be present depending on the severity of right heart decompensation. A chest radiograph may show right heart enlargement and abnormal lung vessels. ECG frequently shows alterations in heart rhythm and changes compatible with right ventricular hypertrophy. Echocardiography is also used to estimate PA pressure based on tricuspid regurgitant jet velocity; however, the accuracy of this estimation is low. Thus, despite echocardiography being a good screening tool to suggest PAH, it should never be used to establish a diagnosis of PAH without proceeding with right heart catheterization. Additionally, laboratory tests should be judiciously applied as indicated by history and physical examination. Chest computed tomography may be warranted to evaluate for interstitial lung disease or emphysema if symptoms or previous radiographs are suggestive. Ventilation–perfusion scan and, if needed, pulmonary angiogram should be performed in those patients with a suggestive history for pulmonary embolism and chronic thromboembolic PH. Overnight oximetry or polysomnography can also be used to exclude obstructive sleep apnea/hypopnea. In excluding alternative diagnoses to PH, serological studies should be ordered for patients with a suspicion of collagen vascular disease, HIV, or hepatitis.

As we mentioned above, right heart catheterization is an absolute requirement for proper evaluation of suspected PAH patients. Diagnosis of PAH by right heart catheterization is confirmed when the mean PA pressure (mPAP) exceeds 25 mm Hg and that PA occlusion pressure (PAOP) or pulmonary capillary wedge pressure is <15 mm Hg. The latter can be measured by passing a balloon-tipped PA catheter from the jugular, femoral, or brachial vein (jugular and femoral approaches are typically used in our institution) through the right atrium and the right ventricle (RV) and into the PA. The catheter is advanced until the balloon tip occludes or wedges into a small PA. The standard PA catheter will measure the pressure at the very tip of the catheter, beyond the balloon, which now blocks the pressure coming from the RV and main PA. In the wedge position, the catheter can measure the reflected pressure coming backward from the end-diastolic left ventricular (LV) pressure, the left atrium, and through the pulmonary veins and capillaries to the PA. It is important to note that PAOP or pulmonary capillary wedge pressure at the end of expiration is measured in all patients. Inferior vena cava occlusion is used in complex cases; however, fluid challenge or exercise helps to diagnose LV dysfunction. If there is further concern for coronary disease or discrepancy from previous right heart catheterization, LV end-diastolic pressure can be measured by left heart catheterization.

When the mPAP is elevated >25 mm Hg at rest or 30 mm Hg with exercise and the PAOP or pulmonary capillary wedge pressure is <15 mm Hg, the vascular resistance is at the pulmonary arterioles and capillaries (eg, precapillary) and this defines patients with PAH. Some definitions have also included pulmonary vascular resistance (PVR) >3.0 Wood units.

If, on the contrary, the PAOP is elevated >15 mm Hg, the pressure elevation comes from the LV or pulmonary veins and is referred to as pulmonary venous hypertension (PVH or group 2; see Figure 1). Pulmonary venous hypertension can occur secondary to systolic or diastolic dysfunction of the LV. The latter is more commonly referred to as heart failure with preserved ejection fraction or nonsystolic heart failure. With chronic elevations in LV pressure, the pulmonary vasculature can vasoconstrict and undergo pathological remodeling, leading to an elevated PVR and high transpulmonary pressure gradient. These patients often exhibit PH out of proportion...
to the degree of LV dysfunction or have persistent PAH after therapies that lower their PAOP, such as diuretics and systemic blood pressure control. These patients exhibit a form of PH, which resembles World Health Organization group 1 PAH, however the prognosis or response to current PAH therapeutics is not known.3

The Physiological Basis of Symptoms in PAH
The symptoms of PAH, such as shortness of breath with exercise or exertion, fluid retention and lower extremity edema, and presyncope/syncope, are almost entirely related to a progressive decline in right heart function, also called right heart failure. This is caused by a progressive increase in afterload.

Figure 1. Clinical classification of pulmonary hypertension from Nice. ALK1 indicates activin-receptor-like kinase-1; BMPRII, bone morphogenetic protein receptor type II; and PAH, pulmonary arterial hypertension.

Figure 2. Algorithm for diagnosis and severity rating of pulmonary hypertension (PH). 6MWD indicates 6-minute walk distance; ANA, antinuclear antibody serology; BNP, brain natriuretic peptide; CHD, congenital heart disease; CT, computed tomography; CTEPH, chronic thromboembolic pulmonary hypertension; CXR, chest x-ray; LFTs, liver function tests; LH, left heart; NT-proBNP, N-terminal fragments of proBNP; PFTs, pulmonary function tests; RH, right heart; RV, right ventricle; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular vplane systolic excursion; TRV, tricuspid regurgitant velocity; VHD, valvular heart disease; and VQ, ventilation-perfusion.
or PVR to a point where the right heart starts to dilate and fail as it is unable to meet the increased demand of the afterload. The RV is a very thin-walled crescent-shaped structure that is designed to pump blood through a low pressure and high-flow pulmonary vascular system. The rapid exchange of carbon dioxide and oxygen between the circulating red blood cells and the air in the alveoli within a brief 1-second transit time requires a very thin sheet of blood exposed to a massive surface area of air. This is accomplished by the alveolar gas exchange unit, the alveolus, which is composed of a 1 cell thin alveolar epithelial cell on the air-environment side and 1 thin endothelial cell on the lung-blood capillary side. The entire cardiac output (CO) of 4 to 6 L of blood flow per minute must spread out over a surface area the size of a tennis court for such efficient gas exchange; this requires a very high flow at a very low pressure to limit alveolar injury and pulmonary edema (fluid leak into the lung). For this reason, the RV is designed to accept a large blood volume (preload) and pump blood against a low resistance (low afterload).

The hemodynamic system can be compared with an electric circuit subject to the Ohm law (V=IR), which states that voltage (V), or the potential difference across a resistor, is proportional to the electric current (I) times the resistance (R). The pulmonary circulation hemodynamics is governed by the same relationship: \( \text{mPAP−PAOP=CO}\times\text{PVR} \), where mPAP−PAOP is the pressure gradient (analogous to voltage or potential across a resistor) and quantifies the gradient across the pulmonary resistance arterioles, CO is analogous to current (I), and PVR is analogous to R. The application of the Ohm law to fluid dynamics is referred to as the Darcy law.

Figure 3 is a classic representation of the natural history of PAH and how the right heart starts to fail as the disease progresses. As the severity of the vascular disease progresses, the effective diameter of the blood vessels decreases, leading to a progressive and irreversible rise in PVR. While the right heart maintains its systolic function and CO, there is a progressive increase in mPAP. As the PVR rises, the right heart eventually begins to fail and CO starts to drop. During severe decompensation with right heart failure (decrease in CO) the mPAP can, paradoxically, drop according to the Ohm law calculation with a very low CO and fixed high PVR. Patients at the highest risk of death are those with evidence of progressive right heart failure, elevated right atrial pressure, low CO, and episodes of systemic hyperperfusion with angina (chest pain) or syncope (sudden loss of consciousness). Sudden death can occur with a profound failure of the RV that leads to inadequate LV filling pressure, resulting in loss of blood flow to the brain, which is followed by cardiac asystole.

Because dyspnea or shortness of breath is primarily caused by an inability to increase CO rather than impaired alveolar gas exchange, the oxygen levels often do not drop until PAH is very advanced and mixed venous oxygen saturations drop. It should be noted that PAH can also develop secondary to advanced lung diseases, such as interstitial lung diseases or chronic obstructive pulmonary disease, in which case gas exchange is impaired early in the course of disease and patients present with more significant hypoxia, such as cyanosis and digital clubbing. Recent mechanistic studies in rodent models with gas exchange imaging in the lung actually suggest that oxygen transfer occurs to some extent across the pulmonary arterioles, suggesting that impaired gas exchange does indeed occur even in PAH without parenchymal lung disease.

Although PAH is characterized by right heart failure, progressive diastolic RV pressure overload can also adversely affect the LV diastolic filling and output. Displacement of the interventricular septum during the diastolic phase with right heart failure can compromise LV filling and volume (referred to as diastolic ventricular interaction or interdependence). A recent study explored this mechanism in non-severe PAH patients (mPAP, 29 mmHg), using invasive pressure–volume loop analysis during simultaneous atrial pacing to a heart rate of 120 bpm. Remarkably, even in these mildly affected patients, stroke volumes dropped by ≥25% during pacing. When the inferior vena cava was occluded to reduce the RV filling transiently, the LV end-diastolic volume increased by 7% and end-diastolic pressures dropped, resulting in an 11% increase in CO. This provided direct evidence that the heart rate–dependent increase in the RV pressure/volume overload adversely impacts the LV cardiac filling and output, even in the setting of normal LV systolic function.

The Genetic Basis of PAH
Although PAH clearly can be inherited in an autosomal dominant mode, it is much more likely that a patient has idiopathic disease, a spontaneous mutation, or PAH developing secondary to another coexistent disease. There are ways to diagnose familial PAH (FPAH, disease that occurs in multiple family members), which include molecular genetic testing for a most common causative of FPAH (bone morphogenetic protein receptor type II [BMPRII]), excluding other known causes of PAH, and the presence of ≥2 family members with PAH. Certainly, more tests and carefully tracking the family medical history can provide accurate diagnosis.

FPAH may show genetic anticipation, with younger age of onset and death in subsequent generations, as well as incomplete penetrance, with only ≥20% of individuals carrying the mutant gene develop the disease. Familial disease is related to germline mutations in genes encoding transforming growth factor-β receptor superfamily, namely endoglin 1, the activin-receptor-like kinase-1, and the BMPRII. A rare new genetic mutation in a low-conductance potassium channel KCNK3 (potassium channel subfamily K member 3), which reduces potassium channel current, has recently been associated with FPAH and IPAH. Mutations in the BMPRII gene have been found in ≥70% patients with FPAH. In addition, 10% to 40% of patients with sporadic IPAH have been found to carry similar mutations. Because of low penetrance of PAH, multiple generations may be unaffected in the family tree and show negative family history of the disease. FPAH and sporadic
IPAH patients with identified genetic mutations are now referred to as heritable PAH.

There are 144 distinct BMPRII mutations found in patients with PAH, which affect multiple loci in the gene, including the ligand-binding domain, the serine-threonine kinase domain, or the cytoplasmic tail. About 70% of mutations are nonsense or frame-shift mutations, which result in nonsense-mediated mRNA decay of the BMPRII mutant transcripts, thus leading to haploinsufficiency. Roughly 30% of mutations have missense cysteine substitutions within the ligand-binding or the kinase domain of BMPRII, which lead to impaired trafficking of BMPRII to the cell membrane. Noncysteine mutations in the kinase domain also affect receptor function. Moreover, BMPRII expression levels are often reduced in patients with PAH independent of BMPRII mutations.

BMP-BMPRII Signaling

BMPs are multifunctional regulators that modulate cell proliferation, differentiation, and apoptosis in different tissues. Under normal conditions, BMPRII, the constitutively active serine-threonine kinase receptor, binds BMPs to form heterotetrameric complexes with one of the type I receptors (ie, BMPRIA, BMPRIB, activin-receptor-like kinase-1, or activin-receptor-like kinase-2), which leads to phosphorylation and activation of the intracellular portion of the type I receptor. Subsequently, the type I receptor phosphorylates ≥1 of the intracellular signal-transduction molecules known as receptor-mediated Smads 1, 5, and 8, which in turn form transcriptional complex with the co-Smad, Smad4. Once formed, the receptor-mediated Smad/Smad4 complex translocates to the nucleus, where it binds to DNA, and interacts with other cofactors to regulate target gene transcriptional responses for either activation or repression (Figure 4).
BMPRII and PAH

Loss of BMPRII in PA endothelial cells increases susceptibility of endothelial cells to apoptosis,17 which leads to endothelial dysfunction and the subsequent development of PAH. Conditional knockout of BMPRII in pulmonary endothelial cells in mice is sufficient for PAH predisposition.18 Moreover, mice with a dominant negative BMPRII mutant in smooth muscle cells develop PAH with pulmonary arterial vascular remodeling.19

Improving BMPRII expression via targeting delivery of wild-type BMPRII to rat lung endothelium has been shown to attenuate PAH.20 Reduced BMPRII expression has also been linked to the activation of microRNAs, including microRNA-20a. Inhibition of microRNA-20a with a specific antagonimiR-20a has been reported to restore BMPRII functional levels in PAs and to prevent vascular remodeling in mice with hypoxia-induced PAH.21 Moreover, enhancement of the cell membrane trafficking of mutant BMPRII using chemical chaperones restores BMP-BMPRII signaling.22

How Common Is PAH?

Data are available from 2 recent observational cohort studies of PAH, one based on 17 university hospitals across France during a 1-year period from 2002 to 2003 (the French Registry), and another one based on 54 centers in the United States (the Registry to Evaluate Early and Long-Term PAH Disease Management [REVEAL Registry]), which enrolled ≈3000 patients with PAH from 2006 to 2007. The estimated incidence and prevalence of PAH are 2.4 and 15 cases per million adults in France, respectively.23 Within the cohort, 39.2% were classified as IPAH and 3.9% were classified as FPAH. In the United States, the estimated incidence and prevalence of PAH are 2.3 and 12.4 cases per million adults, respectively,24 with 46.5% IPAH, and 2.9% of FPAH.25 Despite affecting >3000 patients in the United States, PAH is a rare disease.

PAH can develop in men or women at any age, but it is most common in women. The female-to-male ratio was 1.9:1 in the French Registry.23 Of note, 79% of the adult patients with PAH in the REVEAL Registry were women, with a female-to-male ratio of 4.1:1.24,25 The age of diagnosis is ≈50 years and is similar in both French Registry and REVEAL Registry. The proportions among PAH subgroups in each registry are shown in Figure 5.

Female Sex and PAH

The role of female sex, and specifically estrogenic hormones, in the penetrance, severity, and even treatment of PAH is the subject of active research. However, the findings are not congruent and in fact are paradoxical. For example, there is a female predominance in IPAH and increased penetrance of FPAH in female members of families with mutations in the BMPRII gene.26 On the contrary, 2-methoxyestradiol therapy is protective in monocrotaline-induced PH and female rodents often develop less severe hypoxia-induced RV failure.27 These findings have been referred to as the estrogen paradox and the potential mechanisms are comprehensively reviewed.28 One possible explanation of increased penetrance of FPAH in females...
may relate to the estrogen-metabolizing enzyme, cytochrome p450 1B1.26 Cytochrome p450 1B1 is highly expressed in the lungs, and it can hydroxylate estrogens into 2-hydroxyestrogen, an antimitogenic metabolite that can be further converted to 2-methoxyestradiol to exert protective effects. However, cytochrome p450 1B1 expression levels are 10-fold lower in female BMPRII mutation carriers.29 Estrogens can be hydroxylated into 16α-hydroxyestrone, a mitogenic metabolite that promotes pulmonary vascular remodeling, by other cytochrome p450 enzymes, lowering 2-hydroxyestrogen/16α-hydroxyestrone ratio, thus increasing penetrance of FPAH.26 Further study is clearly required to explain the role of female sex and estrogen in PAH penetrance, prevalence, and severity, as well as the role of 2-methoxyestradiol as a therapeutic.

Mortality and Assessment of Prognosis in PAH
Historically, the National Institutes of Health (NIH) Registry patients had an ≈50% mortality rate at 3 years. Recent clinical trial study groups suggest better outcomes in the modern management era, with observed mortality rates of 20% to 30% at 3 to 5 years in the French Registry and 10% to 30% at 1 to 3 years in the REVEAL Registry. However, this may not be an accurate assessment. This is because less severe patients are enrolled in current clinical trials, with many more patients with a less severe World Health Organization/New York Heart Association functional class II at the time of enrollment. In addition, studies suggest that there are 2 groups of patients with PAH in such trials, each with very different outcomes. One group is described as incident cases. Those are the patients who present at the registration, screening, or clinical trials center for the first time.30 These newly diagnosed patients actually have a mortality rate nearly as high as the original NIH Registry cohort patients. A second group is referred to as prevalent cases, and these patients were diagnosed in the community and then referred to the registration, screening, or clinical trials center.30 These patients have a much better survival rate.31 This may be explained by a lead time diagnosis bias for the second group: sicker patients are more likely to have died before referral in this group, thus selecting for a referred group with better survival. It is noteworthy that REVEAL Registry defined incident cases as patients newly diagnosed within 90 days before enrollment. This can further lead to an immortal time bias that overestimates a better outcome, because death could not have occurred during this time period.32

Hemodynamic Values Define Mortality Risk
Four equations are available to predict a patient’s likelihood of survival according to baseline hemodynamic measurements (Figure 6). The first one was developed in 1981 using the NIH Registry data. In this equation, mPAP, mean right atrial pressure, and cardiac index are important predictors of survival.33 Observed 1-, 3-, and 5-year survival rates for the total cohort were 86%, 69%, and 61%, respectively, which were significantly higher than the predicted survival (65%, 43%, and 32%) based on the NIH Registry equation, further supporting that the NIH equation overestimates mortality rates in PAH.34 Of note, a patient’s response to vasodilatory therapy is included as a survival predictor in the PHC equation. In the modern management era, a third equation was developed using the French Registry. Observed 1-, 2-, and 3-year survival rates (87%, 76%, and 67%, respectively) were improved by ≈20% compared with the classic NIH equation.35 Survival was better in the prevalent cohorts, compared with the incident cohorts, as summarized earlier. In addition, better survival was observed in women and younger patients. Of note, 6-minute walk distance (6MWD), sex, and CO are important parameters applied to the survival equation of the French Registry.36 The most recent equation/risk score calculator was developed based on REVEAL Registry, and a 1-year survival rate from the date of enrollment was 91%, whereas the 1- and 3-year survival rates from the time of PAH diagnosis were 88% and 72%, respectively. The equation is only available for 1-year survival prediction at this time; however, this equation has shown key predictors of survival in the current treatment era. Three key predictors are associated with increased 1-year survival, which are 6MWD ≥440 m, serum brain natriuretic peptide (BNP) level <50 pg/mL, and percent predicted carbon monoxide diffusing capacity ≥280%.

Tests to Evaluate Prognosis
There are many functional, laboratory, and hemodynamic tests that can be performed to refine the risk and prognosis further. These include simple clinical tests such as the 6MWD and heart rate recovery, and biomarker tests such as the BNP or N-terminal fragments of proBNP levels. Imaging studies such as echocardiographic and cardiac MRI are also helpful in prognostication. Measures of right ventricular glycolytic activity may provide new methods to assess clinical risk and are undergoing validation for utilization at this time.

The 6-minute walk test (6MWT) measures simply the distance one can walk in 6 minutes. This test has traditionally served as the work-horse efficacy end point in clinical trials. A normal 6MWD is >600 to 700 m. A distance <300 to 350 m predicts worse outcome in patients with PAH, and a value of <165 m reflects extremely severe limitation.25,35 Although the 6MWD improves with therapy and is typically used as a primary end point for clinical trials of new drugs, the magnitude of improvement in walk distance with therapy that is clinically meaningful remains controversial. In a retrospective analysis of data from the Pulmonary Hypertension Connection (PHIRST) trial, Mathai et al16 used distributional and anchor-based methodologies to conclude that a distance of >33 m is associated with improvement in quality-of-life measures. These results were consistent with a more extensive

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analysis of 10 randomized clinical trials of PAH-targeted therapy, which found that an increase of >42 m best predicted a reduction in the time to clinical worsening. However, in a recent study, it was found that 6MWT in PAH did not accurately demonstrate clinical benefit in outcomes related to active treatments with clear survival benefits. Consideration of other parameters such as heart rate recovery after completion of the 6MWT, defined as the difference in heart rate at the end of 6MWT and at 1 minute after completion of the test, may also be useful to predict outcomes. Another study showed that a heart rate recovery of <16 bpm predicted clinical worsening in 75 consecutive patients with PAH. In this study, heart rate recovery was a better predictor of clinical worsening than 6MWT, but the sample size was very small.

BNP is a hormone, which is released in response to cardiomyocyte stretch, and high levels of which reflect right atrial/ventricular pressure and volume overload. Prognostic importance of BNP has been demonstrated in several cardiovascular disorders. BNP is primarily secreted by the cardiac ventricles as a pro-hormone that is successively cleaved into the N-terminal fragments (N-terminal fragments of proBNP) and the active hormone BNP, both of which are now measured clinically. BNP hormone mediates natriuresis, vasodilation and downregulates the renin–angiotensin–aldosterone axis. Elevated BNP levels predict diminished exercise tolerance and poor prognosis in patients with LV failure. Recently, it has been demonstrated that baseline N-terminal fragments of proBNP can directly correlate with 6MWD. This provides an additional evaluation to be used in clinical trials for PAH. BNP levels are also increased, albeit to lower levels, in patients with primary and severe secondary PH; the degree of elevation reflects the patient’s clinical and hemodynamic status.

Doppler echocardiography provides a noninvasive assessment of the RV structure and function, and it is used to monitor progression and response to therapy. There are many measurements that can be performed using Doppler echocardiography, including RV performance index, RV systolic pressure and right atrial pressure, pericardial effusion, indexed right atrial area, the degree of septal shift toward the LV in diastole, and tricuspid annular plane systolic excursion. The RV performance index serves as a measure of the RV function and was found to be a strong predictor of adverse outcome. The tricuspid regurgitant jet is generally used to estimate the RV systolic pressure by the Bernoulli equation: $4v^2$, where $v$ is the maximum jet velocity of the tricuspid valve. Right atrial pressure is estimated based on the collapsibility of the inferior vena cava, and this measure is added to the peak systolic pressure calculated by the peak tricuspid regurgitant flow to quantify the RV systolic pressure. This measurement approximates the PA systolic pressure, assuming there is no evidence of pulmonary valve stenosis or obstruction. The mPAP can be estimated by measuring the early diastolic component of the pulmonary valve insufficiency jet; however, the accuracy of this measure is low compared with more invasive measures. Echocardiography can also be used to estimate PVR by the ratio of the tricuspid regurgitant jet velocity to the acceleration time of the RV ejection into the PA. Other echocardiographic variables that would suggest the presence of PH independently of tricuspid regurgitation velocity include increased dimensions of right heart chambers, abnormal shape and function of the intraventricular septum, increased RV wall thickness, and a dilated main PA. As the RV dilates and the septal wall becomes flattened and eventually bows, the LV is compressed and paradoxical ventricular septal wall motion is observed.

Cardiac MRI has now become the gold standard for assessing the RV size, volume, CO, PA distensibility, and function. RV mass, which is difficult to quantify by other methods, can also be calculated by multiplying the RV volume by the specific gravity of the myocardium (1.05 g/cm³). Moreover, conventional MRI myocardial tagging and fast-strain-encoded imaging allow direct and accurate measurement of RV remodeling.

Angiography is a tool to assess the degree of peripheral vascular pruning seen as the hallmark of oblitative remodeling of PAH. This direct visualization of the branching pattern and vasculopathy illustrates severity of the disease process but can also be used to evaluate response to therapy. Injection of gadolinium during cardiac MRI broadens the scope of the test to include magnetic resonance angiography. This angiogram shows filling defects as seen with thrombi, an important exclusion necessary in the initial classification of PAH, which would otherwise require a computed tomographic scan with an intravenous contrast and a ventilation–perfusion scan.

Computed tomography–positron emission tomography is a promising new approach that may help in evaluation of the severity of PAH and response to therapy. Computed tomography–positron emission tomography measures a shift from oxidative phosphorylation to glycolytic metabolism using positron emission tomography imaging of $^{18}$F-labeled deoxyglucose (FDG) uptake. FDG uptake via the glucose transporter-1 is increased in the RV of patients with PAH and in cultured pulmonary vascular cells. Marsboom et al evaluated the relationship between FDG uptake, cellular glycolytic activity, and PH severity in monocrotaline and Sugen 5416/hypoxia rat models. FDG uptake increased in the pulmonary vasculature as well as the RV, and the signal in the vasculature was related to increased cellular glycolytic activity in proliferating smooth muscle and endothelial cells. This was characterized by increased glucose transporter-1 expression, inhibition of pyruvate dehydrogenase activity by pyruvate dehydrogenase kinase, and reductions in mitochondrial oxidative phosphorylation. Increase in glycolytic activity correlated closely with disease progression and response to 2 therapies, inhibition of pyruvate dehydrogenase kinase by dichloroacetate, and inhibition of tyrosine kinase by imatinib. Hypoxia-inducible factor-1α was also implicated in the development of the glycolytic phenotype. Clinical utility of measurements of FDG uptake in IPAH remains to be determined.
What Causes PAH to Develop?

In all forms of PH, the progressive vasculopathy is a complex with a broad imbalance of vasodilators, such as nitric oxide (NO) and prostacyclin, and vasoconstrictors, such as endothelin-1 (ET-1) and thromboxane A₂. This condition likely precedes the development of secondary aberrant cellular proliferation. Classic vasodilator systems are dysregulated with decreases in endothelial NO synthase (eNOS) function caused by enzymatic uncoupling, decreases in production of prostacyclin (cyclooxygenase-2 dysfunction), and increased expression and activity of the vasoconstrictor and mitogenic ET-1 signaling system. A fundamental scientific understanding of the critical imbalances of these 3 major pathways, NO, prostacyclin, and ET-1, has led to the rapid clinical development of >10 new major Food and Drug Administration–approved medications for the therapy of PAH; more will be discussed in other reviews in this compendium.

In addition to dysregulation of vasodilators and vasoconstrictor factors, there is a parallel induction of major oxidase enzymes, which will produce reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and peroxynitrite. These oxidases are the subject of current active research and include the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) family, xanthine and aldehyde oxidases, an uncoupled eNOS, and dysfunctional mitochondrial. For example, increased expression of NADPH oxidase isoform 4 (Nox4) in the vasculature of patients with PAH may disrupt canonical NO signaling through many pathways. ROS produced from Nox4 and other oxidases can critically alter the balance of NADP⁺/NADPH and tetrahydrobiopterin (BH₄/BH₂), which maintain the cellular redox balance. An increase in BH₂ levels (or decrease in BH₄/BH₂ ratios) results in an uncoupling of the eNOS enzyme, such that it produces superoxide rather than NO. Such an increase in oxidase expression and ROS generation will enhance vasoconstriction, mitogen activation, and cellular proliferation.

Nitric Oxide

Evidence for a critical role of NO in regulating vasodilation through the primary activation of soluble guanylyl cyclase lead to the award of Nobel Prize in Physiology and Medicine to Robert Furchgott, Louis Ignarro, and Ferid Murad in 1992, and provided the scientific backbone for rapid advances in our understanding of NO biology and related therapeutics in PAH. NO is produced in endothelium by eNOS, which catalyzes the oxidation of L-arginine to produce L-citrulline in the presence of oxygen, NAPDH, and essential cofactors such as BH₄. Once formed, NO diffuses to the underlying vascular smooth muscle, where it binds avidly to soluble guanylyl cyclase. Soluble guanylyl cyclase converts GTP to cGMP, which in turn activates downstream cGMP-dependent protein kinase (protein kinase G) to relax smooth muscle contractile filaments and promote vasodilation. While regulating smooth muscle relaxation, NO also inhibits vascular smooth muscle cell proliferation. In addition, NO decreases platelet aggregation and thrombosis in the blood vessel lumen. These vasodilatory, antiproliferative, and antithrombotic signaling effects of NO maintain normal healthy vascular function.

Although PAH is clearly associated with a reduction in NO bioavailability, this does not always correlate with low levels of eNOS. A general consensus is that patients with PAH have impaired NO production associated with diminished eNOS expression, thus promoting pulmonary vasoconstriction and excessive medial proliferation. Similarly, mice with eNOS deficiency are more susceptible to hypoxia-induced PAH. However, other studies found increased eNOS in the plexiform lesions and suggested that this promoted apoptosis-resistant endothelial cell proliferation. Another recent study showed increased eNOS activation secondary to caveolin-1 deficiency that impaired NO signaling through protein kinase G nitration, thus leading to PAH in mice. A potential solution to this paradox is that while eNOS protein levels may be normal, they exhibit impaired NO synthesis activity caused by eNOS uncoupling, a process by which eNOS produces superoxide instead of NO by transferring electrons from the NOS reductase domain to the oxygenase domain, which are then diverted to molecular oxygen rather than to L-arginine. NO production by eNOS correlates closely with the intracellular concentration of BH₄. Without BH₄ or a decrease in BH₄ availability because of BH₄ oxidation to BH₂, NO production is reduced and superoxide generation is increased as a result of eNOS uncoupling. Reduced BH₄ levels have been shown to impair eNOS function in ischemic hearts, and supplementation of BH₄ decreases ROS-dependent generation of superoxide and corrects eNOS dysfunction. In addition, mice that have BH₄ deficiency predispose to hypoxia-induced PAH. Recent studies suggest that eNOS uncoupling is not simply a consequence of low amount of BH₄, but rather results from a decreased ratio of BH₄:BH₂, or increased BH₂ levels in endothelial cells. Although BH₄ was generally considered an inert product of BH₄ oxidation, studies have shown that BH₄ can compete with BH₄ at the binding site of eNOS with equal affinity and exacerbate eNOS uncoupling. In addition, elevated BH₂ levels were linked to the development of PH in lambs.

An increasing number of translational therapies targeting the NO–soluble guanylyl cyclase–cGMP pathway have been shown effective in the treatment of PAH (Figure 7). These include inhalation of NO, which is Food and Drug Administration–approved for the treatment of persistent PH of newborns. More recently, inhalation of nitrite, a product of NO oxidation that can be reduced back in the lung to form NO, has been shown to reverse established PAH in preclinical models. Nitrite, as well as nitrate, which can be converted to nitrite by oral commensal bacteria, has been shown to inhibit PAH through NO-dependent signaling in preclinical studies. Currently, inhaled nitrite is in a multinational phase II proof-of-concept clinical trial. Other agents, such as BH₄ analogs 6R-BH₄ and L-arginine, have been evaluated in clinical trials with varying levels of success.

To date, the most successful therapeutic approach has been to inhibit phosphodiesterase 5, the enzyme responsible for
the hydrolysis of cGMP. This enzyme provides a constant brake on the vasodilatory and antiproliferative effects of NO in pulmonary vascular smooth muscle cells. Selective phosphodiesterase 5 inhibitors, such as sildenafil and tadalafil, are Food and Drug Administration–approved oral therapies for the treatment of patients with PAH who are functional classes II and III. Randomized clinical trials have shown that both sildenafil and tadalafil improve hemodynamics and 6MWD.87,88

**Prostacyclin**

In 1982, Nobel Prize was awarded to Sune Bergström, Bengt Samuelsson, and John Vane for their discovery of prostacyclin, a potent vasodilator produced from arachidonic acid by the cyclooxygenase pathway and prostacyclin synthase in endothelial cells. Prostacyclin binds to its specific I-prostanoid receptor in the underlying smooth muscle cells to promote relaxation and subsequent vasodilation by activating adenylyl cyclase and increasing intracellular cAMP levels, which in turn activates protein kinase A.79 Through the same pathway, prostacyclin attenuates vascular smooth muscle cell proliferation, inhibits platelet aggregation, and exerts anti-inflammatory and antithrombotic effects.80

The critical role of prostacyclin in the development of PAH is supported by the evidence that mice with prostacyclin receptor deficiency develop severe PAH and vascular remodeling after chronic hypoxia exposure.81 In addition, patients with PAH have reduced production of prostacyclin.82 Furthermore, the expression of prostacyclin receptor and synthase are reduced in patients with PAH.83,84

The use of prostacyclin and its analogs is one of the most successful translational therapies for PAH. Continuous intravenous infusion of epoprostenol, a synthetic prostacyclin, decreases PVR, increases CO, and improves exercise capacity and life expectancy.85 It was the first PAH drug and is approved for the treatment of patients with PAH with functional classes III and IV. Despite its therapeutic efficacy, the shortcomings of epoprostenol, including poor stability, high cost, and the need for continuous intravenous infusion, have urged the development of analogs of prostacyclin. These include treprostinil, iloprost, and beraprost. Clinical trials have shown that both subcutaneous and intravenous of treprostinil improve exercise capacity and hemodynamics.86,87 Inhaled iloprost improves functional class by ≥1 level and exercise capacity by ≥100%.87 Clinical trials of beraprost have shown a significant improvement in 6MWD.88 However, a subsequent randomized control trial in the United States failed to show improvement in 6MWD.89

**Endothelin-1**

ET-1 is a 21-amino acid vasoactive peptide, which is one of the most potent vasoconstrictor molecules in biology.90 It is produced from a 39–amino acid precursor, big ET-1, through endothelin-converting enzymes on the endothelial cell membrane.90 ET-1 acts at 2 different G-protein–coupled receptors: ET₆R and ET₁₅R. ET₁₅R receptors are located predominantly on vascular smooth muscle cells and mediate vasoconstriction, as well as proliferation, hypertrophy, cell migration, and fibrosis. ET₆R receptors are found on both endothelial and smooth muscles. ET₆R activation on smooth muscle cells produces vasoconstriction; however, ET₁₅R activation on endothelial cells leads to vasodilation and antiproliferation by increasing NO and prostacyclin production.91

As PAH progresses, the cellular distribution of the ET-1 receptors changes, with increased expression of both constrictive ET₁R and ET₆R on smooth muscle cells and decreased expression of vasodilatory endothelial ET₁R.92 In animals and patients with PAH, it was reported that ET-1 levels were increased in lungs and in circulation.91,92 In addition, plasma levels of big ET-1 were elevated in patients with PAH.93

Antagonists of ET-1 receptors, such as ambrisentan (ET₁₅R selective) and bosentan (dual ET₁₅R and ET₁₅R), are approved treatments for patients with PAH with functional classes III and IV. Clinical trials have shown that both ambrisentan and bosentan decrease mPAP and PVR, increase cardiac index, and improve exercise endurance.94,95 However, bosentan can produce hepatotoxicity.96 Regular monitoring of liver function is required during therapy. Additionally, inhibition of ET-1 production with endothelin-converting enzyme inhibitor, darglutiril, may provide therapeutic benefit in PAH, although data for its use in PAH are limited.97

It is clear that new drugs targeting the major pathways associated with NO, prostacyclin, and ET-1 remain the most likely candidates to enter clinical practice in the near future. The use of an oral prostanoïd (selexipag), a new dual endothelin antagonist (macitentan) and a soluble guanylate cyclase stimulator (riociguat), led to positive clinical results in 2012.98–100

**ROS and Oxidases in PAH Pathogenesis**

**NADPH Oxidase Isoforms**

Accumulating evidence indicates that increased production of ROS is associated with endothelial dysfunction and subsequent development of PAH.97 All members of Nox family are transmembrane proteins, which generate superoxide by transferring electrons from NADPH in the cytosol to oxygen in the extracellular or intracellular space (depending on the isoform). In the pulmonary vasculature, superoxide generated by Nox enzymes, particularly Nox2 and Nox4, are involved in vascular dysfunction and vascular remodeling induced by hypoxia.101,102 In a mouse model of hypoxia-induced PAH, Nox2 deficiency reduced vasoconstrictor activity and vascular remodeling.103 In addition, small interfering RNA–mediated knockdown of Nox4 or Nox4 inhibitor GKT137831 attenuated vascular remodeling.104,105 A cross-talk between ET-1 and Nox has been reported, which may be explained by the fact that ET-1 stimulates the proliferation of pulmonary arterial smooth muscle cells via activation of Nox-catalyzed ROS production.106 Moreover, superoxide produced by Nox has been shown to impair NO signaling by inducing eNOS uncoupling.107 Understanding of the role of Nox isoforms in PAH pathogenesis may present new opportunities for specific inhibitors, such as the peptidic Nox2 inhibitor Nox2ds.108
Xanthine Oxidase

ROS may be produced by other oxidases, such as xanthine oxidase (XO). Under basal conditions, XO exists primarily in its dehydrogenase form (xanthine oxidoreductase), catalyzing the final 2 steps of purine degradation (hypoxanthine-xanthine-uric acid). Under conditions of hypoxia or inflammation, oxidation of critical cysteine residues or proteolytic cleavage converts xanthine oxidoreductase back to the oxidase form, which in turn transfers substrate-derived electrons to oxygen, generating superoxide and hydrogen peroxide. It has been shown that XO can be activated in endothelial cells, the arteries of patients with PAH, and rodent models of PAH induced by hypoxia. It was also found that treatment of XO inhibitors normalized pulmonary
pressure and limited vascular remodeling in these rodent models. New highly potent XOs inhibitors, such as febuxostat, have been developed for the therapy of gout and may now be tested in human PAH.

Mitochondrial ROS
Mitochondria normally generate ROS as by-products of aerobic metabolism by the electron transport chain enzyme complexes, with diversion of \( \approx 3\% \) of net electron flux to oxygen to form superoxide. Most of the electrons flowing through the respiratory chain are ultimately reduced to water; however, some electrons leak from complexes I and III and react with molecular oxygen to produce superoxide. Superoxide is then converted to hydrogen peroxide by superoxide dismutase 2 in mitochondrial matrix. Hydrogen peroxide can be further reduced to water by catalase or can permeate membranes to regulate downstream signaling pathways and to activate redox-sensitive voltage-gated channel, Kv 1.5. In the case of hypoxic pulmonary vasoconstriction and related hypoxic PH, 2 competing hypotheses for mitochondrial ROS have been advanced. One hypothesis by the Schumacker group suggests that increased mitochondrial ROS production at complex III induces intracellular Ca\(^{2+}\) release, which in turn opens Ca\(^{2+}\) channels in the plasma membrane and activates vasoconstriction. This is supported by recent studies in which complex III activity is blocked by conditional deletion of complex III Rieske iron-sulfur protein, which inhibits hypoxia-related increases in mitochondrial ROS and impairs pulmonary vasoconstriction. On the contrary, Archer and colleagues have suggested that reduced, not increased, mitochondrial ROS levels in PA smooth muscle cells in hypoxia inhibit Kv1.5 channel, which causes membrane depolarization, increases intracellular Ca\(^{2+}\), and subsequently stimulates vasoconstriction and smooth muscle cells proliferation. Although the role and levels of mitochondrial ROS in PAH remain controversial, ROS levels seem to increase in mitochondrial at lower oxygen levels, and decreased expression levels of superoxide dismutase 2 and Kv1.5 channel have been observed in patients with PAH. In addition, activation of Kv1.5 channel by the treatment of dichloroacetate decreases smooth muscle cell proliferation in animals with PAH. Finally, it is becoming increasingly clear that mitochondrial function is impaired in PAH and is associated with enhanced cellular glycolytic activity, suggesting a central role for mitochondrial dysfunction in disease pathology. For a more detailed recent review by our group, see Tabima et al as well as discussions in this review series.

Cellular Changes
In addition to the imbalance of vasoactive mediators, dysfunctional endothelium also releases factors (e.g., serotonin, and FGF-2 [fibroblast growth factor-2]) that initiate abnormal vascular proliferation and remodeling in the PAs. Abnormal smooth muscle cell proliferation is the earliest pathobiological feature of vascular remodeling, which leads to muscularization of peripheral PAs and medial hypertrophy in pulmonary muscular arteries (Figure 8). Increased proliferation and progressive migration of smooth muscle cells, which may be derived from stem cells, fibrocytes, or endothelial cells, into the space between endothelium and the internal elastic lamina, termed intimal fibrosis, also leads to occlusive changes in small PAs. In the late stage of disease progression, disorganized proliferation of apoptosis-resistant endothelial cells, smooth muscle cells, fibroblasts, and macrophages leads to formation of a plexiform lesion, a complex lumen-obliterating lesion that has been found frequently in patients with severe PAH. Additionally, platelet activation, continuous intravascular coagulation, and the development of in situ thrombus further occlude the vessel lumen. Endothelial cell apoptosis in the early phase of PAH has been linked to the loss of small PAs. Moreover, lung vascular remodeling can be associated with abnormal proliferation and differentiation of fibroblasts, increased extracellular matrix deposition, and chronic inflammatory events in the adventitia. Together, these uncontrolled and unpredictable events narrow/obstruct/obliterate PAs, thus leading to PAH.

Summary
PAH is characterized by progressive endothelial dysfunction with low NO and prostacyclin bioavailability, increased ET-1 signaling, enhanced oxidative stress, increased smooth muscle contractility, oxidase activation, mitochondrial dysfunction, and ultimate cellular proliferation and pathologic remodeling of the lung vasculature. The changes in PVR subsequently lead to decreased CO and right heart failure. A loss of blood flow/oxygen delivery because of reduced CO is the primary cause of shortness of breath, rather than impaired alveolar gas exchange and arterial oxygen levels. Although PAH can be inherited (with a higher penetrance in women), the prevalence of the disease is higher for IPAH, spontaneous BMPRII mutations, or PAH secondary to co-existent diseases. According to the French Registry, female patients have \( \approx 18\% \) lower mortality rates than male patients with the same hemodynamic measurements. Although there is still no cure for this disease, prognosis and survival in the present day have improved over the 2 decades ago. Finally, current therapies targeting endothelial function and vasodilation/apoptosis have shown efficacy in decreasing pulmonary pressures, increasing CO and right heart function, augmenting functional class, and improving exercise capacity and quality of life of patients with PAH. Moreover, new therapeutic strategies further enhancing NO signaling, reducing oxidative stress, and enhancing mitochondrial function are being developed.

Acknowledgments
We thank Sergei Snovida for helpful comments on the article, Marta Bueno for analysis of survival equations and calculations, and Elfy Chiang for assistance and production of figures.

Sources of funding
M.T. Gladwin receives research support from NIH grants R01HL098032, R01HL096973, and P01HL103455, the Institute for Transfusion Medicine and the Hemophilia Center of Western Pennsylvania.
A Patient Asks Questions…

Why am I really so short of breath? My oxygen levels are not that low…

This is a common point of confusion for patients. It is true that in most lung diseases, which affect the part of the lung in which the exchange of oxygen to carbon dioxide takes place, the oxygen levels drop. This easily explains the shortness of breath, reflecting the lack of oxygen in the body. In pulmonary arterial hypertension (PAH), this part of the lung tissue is not primarily affected. PAH affects the blood vessels that go through the lung and in most cases the oxygen levels do not decrease. What happens is that the obstruction of the blood vessels by the overgrowth of the cells in the vessel wall poses a strain in the right ventricle (RV), that is, the heart chamber that pushes the blood through the lung. This chamber is thick walled and quickly fails in response to the extra work. As a result, its pumping efficiency drops and the amount of blood that the right chambers eject with each contraction (ie, the ejection fraction of the RV) decreases. The RV pushes the blood to the lung but is still a part of the whole circuit of the blood circulation. Therefore, the decreased pumping efficiency of the RV results in a decreased amount of blood circulating in the whole body. Thus, patients with PAH get short of breath not because the oxygen levels in the blood drop but because the amount of the blood (which carries the oxygen) decreases with each heart contraction. The tissues sense the lack of oxygen because they receive less blood.

This is more prominent during increased activity (exercise) when the tissues need more oxygen/blood to cover the increased need for energy (which is offered by the oxygen). This is why patients with PAH are mostly short of breath with activity and only at the very advanced/late stages of the disease they get short of breath at rest. Although oxygen generally makes most people feel better, oxygen supplementation is not critical for the management of PAH, in contrast to most other lung diseases. This also explains why patients may pass out during exercise. The decrease in the amount of blood sent to the brain drops significantly during exercise, causing a transient loss of consciousness (ie, syncope). This concept is important for the patients to understand. It also makes it clear that PAH is essentially a disease of heart failure; only that in contrast to the left chambers affected in the more common forms of heart failure, in PAH only the right chambers are affected.

How long do I have to live?

This is almost an impossible question to answer in Medicine, yet a very important one, bothering most patients. Based on the above discussion, it is clear that the function of the right chambers of the heart are the most important in terms of symptoms (like shortness of breath) in patients with PAH. We now know that the function of the RV is most critical for the survival of patients with PAH as well. However, we do not know what makes the right chambers transition from a state of compensation to a state of failure. We do recognize that there are probably genetic and other reasons that may explain why the RV in one patient may fail earlier than in another patient. Thus, overall, it is premature to claim that we can predict how long a patient has to live, assuming he or she is offered all the available therapies at this point.

However, studies in large numbers of patients with PAH have allowed scientists to develop mathematical equations to predict survival, based on certain parameters, such as the blood pressure in the lung, the cardiac output (ie, the amount of blood ejected from the right chambers), and others. These equations were developed at different points of time, when the management of patients with PAH was different, explaining some differences in the results from these formulas. The authors present in Figure 6 the results for our patient (as described in the introductory article by Dr Michelakis) based on several of these formulas. Using the specific information for our patient, one can see how the 1-year survival is calculated based on formulas developed in the 1980s (such as the NIH formula) to more recent ones that included additional parameters in the formula. Thus, although the calculation from the NIH formula would have predicted a 75% chance of our patient to survive 1 year, more recent formulas predict a higher chance, ~90%. It is important for the patients to understand the efforts of physicians and scientists in the field to provide tools to help in these complicated questions, providing answers that are as specific and individualized as possible.

For the case description, see introductory article by E.D. Michelakis, page 109.

Disclosures

M.T. Gladwin is listed as a coinventor on an NIH government patent for the use of nitrite salts in cardiovascular diseases. M.T. Gladwin consults with Aires Pharmaceuticals on the development of a phase II proof-of-concept trial using inhaled nitrite for PAH. The other authors report no conflicts.

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Pulmonary Arterial Hypertension: The Clinical Syndrome
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Circ Res. 2014;115:115-130
doi: 10.1161/CIRCRESAHA.115.301146
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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