Pulmonary arterial hypertension: pathogenesis and clinical management

Thenappan Thenappan,¹ Mark L Ormiston,² John J Ryan,³ Stephen L Archer²

ABSTRACT

Pulmonary hypertension is defined as a resting mean pulmonary artery pressure of 25 mm Hg or above. This review deals with pulmonary arterial hypertension (PAH), a type of pulmonary hypertension that primarily affects the pulmonary vasculature. In PAH, the pulmonary vasculature is dynamically obstructed by vasoconstriction, structurally obstructed by adverse vascular remodeling, and pathologically non-compliant as a result of vascular fibrosis and stiffening. Many cell types are abnormal in PAH, including vascular cells (endothelial cells, smooth muscle cells, and fibroblasts) and inflammatory cells. Progress has been made in identifying the causes of PAH and approving new drug therapies. A cancer-like increase in cell proliferation and resistance to apoptosis reflects acquired abnormalities of mitochondrial metabolism and dynamics. Mutations in the type II bone morphogenetic protein receptor (BMPR2) gene dramatically increase the risk of developing heritable PAH. Epigenetic dysregulation of DNA methylation, histone acetylation, and microRNAs also contributes to disease pathogenesis. Aberrant bone morphogenetic protein signaling and epigenetic dysregulation in PAH promote cell proliferation in part through induction of a Warburg mitochondrial-metabolic state of uncoupled glycolysis. Complex changes in cytokines (interleukins and tumor necrosis factor), cellular immunity (T lymphocytes, natural killer cells, macrophages), and autoantibodies suggest that PAH is, in part, an autoimmune, inflammatory disease. Obstructive pulmonary vascular remodeling in PAH increases right ventricular afterload causing right ventricular hypertrophy. In some patients, maladaptive changes in the right ventricle, including ischemia and fibrosis, reduce right ventricular function and cause right ventricular failure. Patients with PAH have dyspnea, reduced exercise capacity, exertional syncope, and premature death from right ventricular failure. PAH targeted therapies (prostaglandins, phosphodiesterase-5 inhibitors, endothelin receptor antagonists, and soluble guanylate cyclase stimulators), used alone or in combination, improve functional capacity and hemodynamics and reduce hospital admissions. However, these vasodilators do not target key features of PAH pathogenesis and have not been shown to reduce mortality, which remains about 50% at five years. This review summarizes the epidemiology, pathogenesis, diagnosis, and treatment of PAH.

Introduction

Pulmonary hypertension is defined as a resting mean pulmonary artery pressure (mPAP) of 25 mm Hg or above. The classification system proposed by the Fifth World Symposium on Pulmonary Hypertension attempts to guide the clinical approach to pulmonary hypertension by dividing patients into five groups: group 1—pulmonary hypertension due to pulmonary vascular disease; group 2—pulmonary hypertension due to left heart disease; group 3—pulmonary hypertension due to lung disease or hypoxia; group 4—pulmonary hypertension due to chronic thromboembolic disease; and group 5—a miscellaneous collection of pulmonary hypertension syndromes caused by a variety of disorders, including hemolytic anemias and sarcoidosis (fig 1).¹ In principle, patients in each of these groups share pathophysiology, prognosis, and therapeutic response; in reality, tremendous heterogeneity exists within each group.
This review focuses on group 1 pulmonary hypertension, also known as pulmonary arterial hypertension (PAH). PAH is a disease of the cardiopulmonary unit, affecting the pulmonary arterial and venous circulation and the right ventricle. Obstructive, hyperproliferative, vascular lesions, vasoconstriction of pre-capillary arterioles, and venous obstruction (in some forms of group 1 disease) increase pulmonary vascular resistance (PVR), increase right ventricular afterload, and promote right ventricular failure (RVF), which is the leading cause of death in PAH. Current treatments for PAH are primarily pulmonary vasodilators. They ameliorate symptoms and reduce hospital admissions, but they are expensive and not curative.

This review summarizes the epidemiology, diagnostic evaluation, and treatment of PAH. It also examines recent advances in basic science, noting potential therapeutic targets and future research questions.

**Sources and selection criteria**

We identified references for this review by doing a PubMed search for years 2007-17. We only included peer reviewed articles written in English. We used the following search terms in combination with the term “pulmonary hypertension”: “mechanisms”, “experimental models”, “diagnosis”, “epidemiology”, “survival”, “pulmonary arterial hypertension”, “guidelines”, “classification”, “imaging”, “hemodynamics”, and “therapy”. We included articles on the basis of the quality of study design and size, favoring randomized controlled trials, reports from large registries, and guidelines. For the pathogenesis section, we selected papers in reputable journals and highlighted evidence in which concordant data were available from more than one research group. We also included highly cited papers written before 2007. We excluded case reports and papers in non-peer reviewed journals. We screened approximately 1000 articles of evidence classes I-IV and included about 740 for detailed review.

**Epidemiology and natural history of PAH**

The incidence of PAH ranges from 2.0 to 7.6 cases per million adults per year, and its prevalence varies from 11 to 26 cases per million adults (table 1). The incidence of PAH is fourfold higher in women than in men, but survival is paradoxically worse in men with PAH. Nearly half of the patients have idiopathic PAH (IPAH), heritable PAH, or anorexigen induced PAH, with connective tissue disease associated PAH (APAH) being the second most common subgroup. The National Institutes of Health (NIH) registry from the 1980s was the first major epidemiological study of PAH. Subsequently, 10 major registries have described the epidemiology of PAH (table 1). These modern registries have provided two novel insights. Firstly, in the current era, patients with IPAH, heritable PAH, or anorexigen associated PAH are older than those in the NIH registry (mean age at diagnosis 45-65 versus 36 years). Potential explanations include increased awareness of PAH due to the availability of PAH specific therapies and widespread use of Doppler echocardiography, leading to wider recognition of PAH in older patients.


<table>
<thead>
<tr>
<th>State of the Art Review</th>
<th>Pulmonary arterial hypertension</th>
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<tr>
<td>1.1 Idiopathic</td>
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<td>1.2 Heritable</td>
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<td>1.2.1 BMP2R2 mutation</td>
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<td>1.2.2 Other</td>
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1. Persistent pulmonary hypertension of the newborn

<table>
<thead>
<tr>
<th>Pulmonary hypertension due to left heart disease</th>
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<tr>
<td>2.1 Left ventricular systolic dysfunction</td>
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<th>Pulmonary hypertension due to lung diseases and/or hypoxia</th>
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<td>3.1 Chronic obstructive pulmonary disease</td>
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<td>3.2 Interstitial lung disease</td>
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<td>3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern</td>
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<td>3.4 Sleep disordered breathing</td>
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<td>3.5 Acute respiratory disorders</td>
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<td>3.6 Chronic exposure to high altitude</td>
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<td>3.7 Developmental lung disease</td>
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<tr>
<th>Chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions</th>
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<td>4.1 Chronic thromboembolic pulmonary hypertension</td>
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<tr>
<td>4.2 Other pulmonary artery obstructions</td>
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<td>4.2.1 Angiosarcoma</td>
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<td>4.2.2 Other intravascular tumors</td>
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<tr>
<td>4.2.3 Arteritis</td>
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<tr>
<td>4.2.4 Congenital pulmonary arteries stenosis</td>
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<td>4.2.5 Parasites (hydatidosis)</td>
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<th>Pulmonary hypertension with unclear and/or multifactorial mechanisms</th>
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<tr>
<td>5.1 Hematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy</td>
</tr>
<tr>
<td>5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis</td>
</tr>
<tr>
<td>5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders</td>
</tr>
<tr>
<td>5.4 Others: pulmonary tumoral microangiopathy, fibrosing mediastinitis, chronic renal failure (with/without dialysis), segmental pulmonary hypertension</td>
</tr>
</tbody>
</table>

This review summarizes the epidemiology, diagnostic evaluation, and treatment of PAH. It also examines recent advances in basic science, noting potential therapeutic targets and future research questions.
However, as the mean age of the group 1 cohort increases, the risk of misdiagnosis increases, as group 2 pulmonary hypertension in patients with heart failure and preserved ejection fraction (HFpEF) is much more prevalent and shares several echocardiographic abnormalities.

Secondly, long term survival in patients with PAH has significantly improved in the past two decades. The median survival is now six years, compared with 2.8 years in the 1980s.13 Similarly, one year survival rates for PAH patients range from 86% to 90%, improved from 65% in the 1990s.14-16 Improved awareness of PAH, availability of PAH specific therapies, long term anticoagulation therapy, and better management of RVF are possible reasons for improved survival.

Nonetheless, PAH still causes a substantial clinical and economic burden. Although PAH related hospital admissions decreased between 2001 and 2012, the mean charge and length of stay per PAH related admission have increased with no significant decline in inpatient mortality.17

Diagnosis of PAH
PAH is defined as a resting mPAP of 25 mm Hg or above, pulmonary capillary wedge pressure (PCWP) below 15 mm Hg, and PVR above 3 Wood units18), in the absence of more prevalent causes of pulmonary hypertension such as left heart disease, chronic lung disease,19 or venous thromboembolism.

Most patients are referred for evaluation for pulmonary hypertension after detection of increased right ventricular systolic pressure (RVSP) by Doppler ultrasound. RVSP is calculated from Bernoulli’s principle, on the basis of the velocity of the tricuspid regurgitant jet (RVSP=4V2, where V is the maximum tricuspid regurgitant jet velocity) plus the estimated right atrial pressure (RAP). RAP is determined by observing the jugular venous pressure or, more commonly, by assessing the dimensions of the inferior vena cava, at baseline and in response to snifing. On the basis of comparison with right heart catheterization (RHC), an inferior vena cava maximum diameter above 19 mm or a collapse of 30% or less on sniff testing implies an RAP above 10 mm Hg.22 The tricuspid regurgitant estimate of RVSP can underestimate or overestimate pulmonary artery systolic pressure and does not measure mPAP or indicate whether the pulmonary hypertension relates to intrinsic pulmonary vascular disease.23

Hence, echocardiographic findings suggestive of pulmonary hypertension should prompt referral to specialized centers for further evaluation to confirm the hemodynamic abnormality and classify the patient into the correct pulmonary hypertension group.24 This is important, as treatment and prognosis vary considerably by the cause of pulmonary hypertension. Figure 2 summarizes the diagnostic algorithm for PAH. Pulmonary function tests and overnight oximetry are needed to evaluate for chronic hypoxic lung disease. The ventilation/perfusion scan is the diagnostic test of choice for excluding chronic pulmonary thromboembolic pulmonary hypertension (CTEPH), as it is more sensitive than a computed tomography pulmonary angiogram in identifying distal CTEPH.25 An invasive pulmonary angiogram or computed tomography angiogram of the chest may be done for patients with a high probability ventilation/perfusion scan for confirmatory diagnosis. Exclusion of CTEPH is important, as it can be cured by surgical pulmonary thromboendarterectomy in suitable candidates. Serological testing helps to identify associated causes of PAH, such as connective tissue disease or cirrhosis. RHC is mandatory for confirming the diagnosis of PAH and is required before PAH targeted treatment is started. RHC also assesses the severity of pulmonary hypertension, evaluates potential left heart disease, and identifies the

### Table 1 | Characteristics of pulmonary arterial hypertension (PAH) registries

<table>
<thead>
<tr>
<th>Registry</th>
<th>Date of enrolment</th>
<th>Sample size, No</th>
<th>Patient population, %</th>
<th>Mean (SD) age, years</th>
<th>Incidence and prevalence</th>
<th>One year survival, %</th>
<th>Five year survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH3</td>
<td>1981-88, prospective</td>
<td>187</td>
<td>IPAH, heritable; drug induced</td>
<td>36 (15)</td>
<td>NA</td>
<td>68</td>
<td>34</td>
</tr>
<tr>
<td>US-PHC4</td>
<td>1982-2006, retrospective</td>
<td>578</td>
<td>IPAH: 44; CTD: 30; CHD: 11; POPH: 7; heritable: 4; anorexigen: 3; HIV: 1</td>
<td>48 (14)</td>
<td>NA</td>
<td>86</td>
<td>61</td>
</tr>
<tr>
<td>Scottish5</td>
<td>1986-2001, retrospective</td>
<td>374</td>
<td>IPAH: 47; CTD: 30; CHD: 23</td>
<td>Men 50 (13); women 52 (12)</td>
<td>PAH incidence 7.6 cases/MAI/year and prevalence 26 cases; IPAH incidence 2.6 cases/MAI/year and prevalence 9 cases/MAI</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mayo Clinic6</td>
<td>1995-2004, prospective</td>
<td>484</td>
<td>IPAH/heritable: 56; CTD: 24; POPH: 10.4; CHD: 9.2; HIV: 0.4</td>
<td>52 (15)</td>
<td>NA</td>
<td>81.1</td>
<td>47.9</td>
</tr>
<tr>
<td>Chinese7</td>
<td>1999-2004, prospective</td>
<td>72</td>
<td>IPAH: 94.4; heritable: 5.6</td>
<td>35.9 (12.2)</td>
<td>NA</td>
<td>68</td>
<td>20.8</td>
</tr>
<tr>
<td>French8</td>
<td>2002-03, prospective</td>
<td>674</td>
<td>IPAH: 39.2; CTD: 15.3; CHD: 11.3; anorexigen: 9.5; HIV: 6.2; heritable: 3.9; POPH: 0.4</td>
<td>50 (15)</td>
<td>PAH incidence 2.4 cases/MAI/year and prevalence 15 cases/MAI; IPAH incidence 1.0 cases/MAI/year and prevalence 5.9 cases/MAI</td>
<td>87</td>
<td>NA</td>
</tr>
<tr>
<td>REVEAL9</td>
<td>2006-07, prospective</td>
<td>2525</td>
<td>IPAH: 46.2; CTD: 25.3; CHD: 9.9; POPH: 5.3; heritable: 5.2; anorexigen: 2.9; HIV: 1.9</td>
<td>53 (14)</td>
<td>PAH incidence 2.0 cases/MAI/year and prevalence 10.6 cases/MAI; IPAH incidence 0.9 cases/MAI/year</td>
<td>Incident: 86.3; prevalent: 90.4</td>
<td>Incident: 61.2; prevalent: 65.4</td>
</tr>
<tr>
<td>UK and Ireland10</td>
<td>2001-09, prospective</td>
<td>482</td>
<td>IPAH: 93; heritable: 5; anorexigen: 2</td>
<td>50 (17)</td>
<td>Incidence 1.1 cases/MAI/year and prevalence 6.6 cases/MAI</td>
<td>93</td>
<td>60</td>
</tr>
<tr>
<td>New Chinese registry11</td>
<td>2008-11, prospective</td>
<td>956</td>
<td>CHD: 4.3; IPAH: 35; CTD: 19</td>
<td>36 (13)</td>
<td>NA</td>
<td>PAH: 92.1; CTD: 85.4</td>
<td>NA</td>
</tr>
<tr>
<td>COMPERA12</td>
<td>2007-11, prospective</td>
<td>587</td>
<td>IPAH: 97; anorexigen: 2; heritable: 1</td>
<td>71 (16)</td>
<td>NA</td>
<td>92</td>
<td>NA</td>
</tr>
</tbody>
</table>

CTD=connective tissue disease; CTD=congenital heart disease; PAH=pulmonary arterial hypertension; MAI=million adult inhabitants; NIH=National Institutes of Health; POPH=pulmonary hypertension; US-PHC=United States Pulmonary Hypertension Connections.
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Among the most common challenges in clinical practice is distinguishing between PAH and group 2 pulmonary hypertension secondary to HFpEF. Both syndromes have normal left ventricular systolic function and abnormal left ventricular diastolic parameters. Correct case classification is crucial, as HFpEF accounts for a considerable proportion of referrals to pulmonary hypertension centers, and PAH specific therapies are not effective in group 2 pulmonary hypertension.

Fig 2 | Diagnostic testing algorithm for pulmonary arterial hypertension. 6MWT=six minute walk test; ABGs=arterial blood gases; ANA=antinuclear antibody serology; BNP=brain natriuretic peptide; CBC=complete blood count; COPD=chronic obstructive pulmonary disease; CPET=cardiopulmonary exercise test; CT=computed tomography; CTD=connective tissue disease; CXR=chest x ray; DLCO=diffusion capacity of the lungs for carbon monoxide; ECG=electrocardiogram; LHD= left heart disease; HRCT=high resolution computed tomography of the chest; LFT=liver function tests; MRI=magnetic resonance imaging; PA=pulmonary artery; PAP/CO=pulmonary artery pressure and cardiac output; PEA=pulmonary endarterectomy; PFT=pulmonary function tests; PH=pulmonary hypertension; RV=right ventricle; RVE=right ventricular enlargement; TEE=transesophageal echocardiogram; TR=tricuspid regurgitation; Tx=treatment; V/Q scan=ventilation/perfusion scintigram. This figure is modified to reflect the authors’ practice but was based on a figure in McLaughlin VV, et al. *J Am Coll Cardiol* 2009;53:1573-1619.

Differentiating PAH from pulmonary hypertension due to HFpEF

Among the most common challenges in clinical practice is distinguishing between PAH and group 2 pulmonary hypertension secondary to HFpEF. Both syndromes have normal left ventricular systolic function and abnormal left ventricular diastolic parameters. Correct case classification is crucial, as HFpEF accounts for a considerable proportion of referrals to pulmonary hypertension centers, and PAH specific therapies are not effective in group 2 pulmonary hypertension. Two metrics that differentiate the HFpEF group are increased left atrial size on echocardiography and increased PCWP on RHC. In normal older patients the mean PCWP is 9 (SD 3) mm Hg in men and 11 (3) mm Hg in women. Including patients with PCWP values above 15 mm Hg in the group 1 category is thus inappropriate. Skilled measurement of PCWP is critical to distinguish between group 2 pul-
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Differentiating PAH from pulmonary hypertension due to chronic lung disease or hypoxia

Table 2 lists the characteristics that can help physicians in differentiating PAH from group 3 pulmonary hypertension due to chronic lung disease. This is important, as PAH specific therapies have not been shown to be effective in pulmonary hypertension due to lung disease.

Assessing right ventricular function

Right ventricular function is the major determinant of long term outcomes in PAH. Several echocardiographic parameters that reflect increased right ventricular and right atrial size or decreased right ventricular contractility are better prognostic indicators in PAH than RVSP.
Increased mortality risk is predicted by reduced right ventricular systolic and diastolic function, the presence of pericardial effusion, increased right ventricular or right atrial size, decreased tricuspid annular plane systolic excursion (TAPSE) (fig 6A), decreased Tei index (fig 6B), 40 reduced peak systolic tricuspid lateral annular velocity (S') (fig 6C), 41,42 decreased isovolumic contraction velocity (fig 6D), 43–46 and abnormal right ventricular free wall strain.

Unlike the left ventricle, the right ventricle shortens from apex to base with contraction. TAPSE assesses longitudinal right ventricular shortening by measuring annular excursion in the apical four chamber view, using M mode ultrasound (fig 6A). A TAPSE below 18 mm predicts increased mortality in PAH. 47 Although convenient and reproducible, TAPSE measures only basal right ventricular systolic function and is not as accurate a measure of global right ventricular function as cardiac magnetic resonance imaging (MRI) or three dimensional echocardiography. 48 TAPSE can be influenced by left ventricular function. 49,50 In addition, the timing of the deterioration in TAPSE relative to the onset of RVF is poorly defined. 51

The geometry of the right ventricle and its heavy trabeculation make two dimensional transthoracic estimation of right ventricular function and volume difficult. Three dimensional echocardiography has improved the ability to accurately assess the right ventricle. 52 Right ventricular volume acquisitions and right ventricular ejection fraction (RVEF) measurements using three dimensional echocardiography are accurate and reproducible with minimal inter-observer variability (approximately 4%). 53 Changes in right ventricular volumes and function measured by three dimensional echocardiography correlate with clinical outcomes in PAH. 54 However, because of its low measurement variability, cardiac MRI is more accurate and reproducible than either the two or three dimensional transthoracic technique. For example, to detect a change of 5% in RVEF in a clinical trial population, two and three dimensional echocardiography would require twice and 2.5 times the patient sample size needed for cardiac MRI. 55

Current treatments for PAH
The current treatment strategy for PAH can be broadly divided into general measures, supportive therapies (box 1), and PAH specific therapies.

PAH specific therapies
Fourteen PAH specific therapies are available for PAH. They target components of four PAH relevant molecular pathways: voltage gated, L type calcium channels, nitric oxide cyclic guanosine monophosphate (cGMP), endothelin, and prostacyclin. These drugs do not directly target the adverse vascular remodeling that obliterates, obstructs, and stiffens the pulmonary vasculature, and most do not improve the function of the right ventricle.

The vasodilator therapies may indirectly inhibit cell proliferation and regress adverse vascular remodeling when...
Box 1 | General supportive measures and background therapies in PAH management

General supportive measures
Restrict salt and fluid intake to reduce volume overload in light of their limited right ventricular reserve
Supplemental oxygen therapy should be used if needed to maintain systemic oxygen saturation above 90% as it improves exercise capacity
Exercise training is advocated in PAH. Exercise training increases six minute walk distance (+52 m), peak oxygen uptake, and quality of life in PAH. Low level, symptom limited, aerobic exercises are preferred, and intense isometric exercises can lead to syncope
Vaccination against influenza and pneumococcal pneumonia is recommended
Women in the reproductive age group are strongly counseled to use contraceptive measures

Background therapies for PAH
Diuretics—Diuretics are used to treat venous congestion resulting from RVF
Digoxin—Digoxin improves right ventricular contractility and cardiac output in acute hemodynamic studies; however, no data support its long term use in PAH. The benefits and risks should be weighed
Anticoagulation—In situ thrombosis occurs in the small resistance pulmonary arteries of PAH patients. Retrospective and prospective studies have reported improved survival with long term warfarin therapy in PAH. Registry data have questioned the benefits of anticoagulation, especially in patients with APAH. In the COMPERA registry, the use of anticoagulation was associated with a 21% improvement in survival in patients with IPAH but no benefit in those with APAH. The REVEAL registry found no survival benefit with anticoagulation in IPAH and reduced survival in APAH. Long term warfarin therapy is recommended only in IPAH, heritable PAH, or anorexigen associated PAH patients with an international normalized ratio goal of 1.5-2.5. The role of novel oral anticoagulants is unknown

Drugs targeting the nitric oxide pathway
Nitric oxide is a potent pulmonary vasodilator that activates soluble guanylate cyclase (sGC) to generate cGMP. cGMP causes pulmonary artery smooth muscle cell (PASM) relaxation through cGMP dependent protein kinase, which activates soluble guanylate cyclase (sGC) to generate cGMP.

Calcium channel blockers
Calcium channel blockers (CCBs) are effective in the 5-10% of PAH patients who respond to acute vasodilatory challenge during RHC with a drop in mPAP by between 10 and 40 mm Hg, with no drop in cardiac output. Inhaled nitric oxide, intravenous epoprostenol, or intravenous adenosine are used for acute vasodilator testing. Vasoreactivity may be due to genetic predisposition, resulting from enrichment in vascular smooth muscle cell contraction gene variants. Early studies suggest that vasoreactivity can potentially be identified on the basis of a simple peripheral blood mRNA expression profile (reduced levels of mRNA for DSG2, a desmosomal cadherin, and RHOQ, a cytoskeletal protein).

CCBs are indicated only in PAH patients with a documented, positive vasodilator test. These patients are uncommon (5-10% of all cases) and have a different natural history with a five year survival rate of 90% with CCB monotherapy. Eligible PAH patients generally need higher than usual doses of CCB: amiodopine 20 mg, nifedipine 120-240 mg, or diltiazem 240-720 mg daily. Verapamil is not used because of its negative inotropic effects. Although diltiazem can also have a negative inotropic effect, it is preferred over amiodopine or nifedipine in patients with sinus or atrial tachycardia. Patients treated with CCBs should be closely monitored for adequate response and transitioned to PAH specific therapies if symptoms progress. Adequate long term response to CCBs in patients with APAH is rare.

Table 2 | Characteristics differentiating pulmonary arterial hypertension from group 3 pulmonary hypertension

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pulmonary arterial hypertension</th>
<th>Group 3 pulmonary hypertension</th>
</tr>
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<tbody>
<tr>
<td>Ventilatory function:</td>
<td>FEV1 (in COPD)</td>
<td>≥60% predicted</td>
</tr>
<tr>
<td></td>
<td>FVC (in ILD)</td>
<td>≥70% predicted</td>
</tr>
<tr>
<td></td>
<td>TLC (in ILD)</td>
<td>≥60% predicted</td>
</tr>
</tbody>
</table>

| High resolution CT scan of chest | Absence of or only modest airway or parenchymal abnormalities | Characteristic airway and/or parenchymal abnormalities |

<table>
<thead>
<tr>
<th>Cardiopulmonary exercise testing</th>
<th>Circulatory limitations</th>
<th>Ventilatory limitations</th>
</tr>
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<tbody>
<tr>
<td>Preserved breathing reserve</td>
<td>Reduced breathing reserve</td>
<td></td>
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<tr>
<td>Reduced oxygen pulse</td>
<td>Normal oxygen pulse</td>
<td></td>
</tr>
<tr>
<td>Low CO/VO2 slope</td>
<td>Normal CO/VO2 slope</td>
<td></td>
</tr>
<tr>
<td>No change or decrease in PaCO2 during exercise</td>
<td>Increase in PaCO2 during exercise</td>
<td></td>
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<tr>
<td>Mixed venous oxygen saturation at lower limit</td>
<td>Mixed venous oxygen saturation above lower limit</td>
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</table>

CO=cardiac output; COPD=chronic obstructive pulmonary disease; CT=computed tomography; FEV1=forced expiratory volume in 1 sec; FVC=forced vital capacity; ILD=interstitial lung disease; PaCO2=partial pressure of carbon dioxide in arterial blood; TLC=total lung capacity; VO2= oxygen consumption.
walk distance (6MWD), World Health Organization functional class, and mPAP at all three doses studied (20 mg, 40 mg, and 80 mg three times a day) (table 3). It had no effect on clinical worsening. Sildenafil is approved at 20 mg three times a day for treatment of PAH. However, long term extension studies showed significant improvement in hemodynamics with higher doses. Tadalafil (40 mg daily) increases 6MWD, time to clinical worsening, and health related quality of life (table 3). The major side effects of PDE5 inhibitors include headache, flushing, dyspepsia, and epistaxis.

**sGC stimulators**—sGC is a heterodimeric enzyme that generates cGMP. In PAH, sGC is often dysfunctional because it is oxidized or has lost its heme group. Riociguat directly stimulates sGC independent of nitric oxide, resulting in increased cGMP and pulmonary vasodilation. Riociguat 2.5 mg three times a day improves 6MWD, PVR, serum N terminal pro B type natriuretic peptide (NT-proBNP) concentrations, time to clinical worsening, and WHO functional class (table 3). The improvement in 6MWD is observed both in treatment-naive patients and those taking other PAH specific therapies. sGC and PDE5 inhibitors should not be given concurrently owing to the risk of hypotension. However, transition to sGC from PDE5 inhibitors improves exercise capacity and hemodynamics in patients who have inadequate responses to PDE5 inhibitors. Headache, dizziness, hypotension, dyspepsia, and gastroesophageal reflux are the most common adverse effects of riociguat.

**Drugs targeting the endothelin pathway: endothelin receptors antagonists**

Endothelin is a potent vasoconstrictor and smooth muscle mitogen. It acts through endothelin A and endothelin B receptors. Endothelin is overexpressed in the lungs and plasma of patients with PAH. The endothelial receptor antagonists (ERAs) bosentan, ambrisentan, and macitentan are beneficial in PAH.

Bosentan and macitentan are dual ERAs, blocking both endothelin A and endothelin B receptors. Bosentan 125 mg twice a day improves 6MWD, WHO functional class, Borg dyspnea score, and time to clinical worsening (table 3). Bosentan also improves PVR and 6MWD.

Fig 6 | Echocardiographic measures of right ventricular function. (A) TAPSE. M mode cursor placed through right ventricular (RV) apex to lateral tricuspid annulus in apical four chamber view for purpose of measuring distance traveled by annulus in centimeters from end diastole to end systole. Abnormal TAPSE of 1.3 cm is noted by crosshatches. (B) Right ventricular myocardial performance index (RVMPI—Tei index) is defined as sum of isovolumic contraction (IVCT) and isovolumic relaxation (IVRT) time divided by ejection time (ET). Above is representation of two ways to calculate RVMPI, on tissue Doppler and on pulsed wave Doppler. Below are IVCT, IVRT, and ET where RVMPI=(IVCT+IVRT)/ET. (C) Doppler tissue imaging (DTI) of tricuspid annulus. S’ is highest systolic velocity measured by pulsed DTI of tricuspid annulus. Isovolumic contraction velocity (IVCv) is defined as peak velocity by DTI measurement at level of tricuspid annulus in early systole when right ventricle contracts and pressures acutely rise without any change in ventricular volume. These measurements can be done after high frame rate acquisition with color coded Doppler offline (not shown).
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Table 3 | Landmark clinical trials in pulmonary hypertension

<table>
<thead>
<tr>
<th>Date</th>
<th>Interventions</th>
<th>Characteristics</th>
<th>Study cohort</th>
<th>Primary outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>N epoprostenol v placebo</td>
<td>12 week, prospective, randomized, open trial</td>
<td>81 patients: IPAH, heritable, drug induced PAH</td>
<td>6MWD, survival</td>
<td>6MWD improved by 32 m (P=0.002); no deaths with epoprostenol but deaths in control group (P=0.003)</td>
</tr>
<tr>
<td>2000</td>
<td>N epoprostenol v placebo</td>
<td>12 week, prospective, randomized, open trial</td>
<td>111 patients: scleroderma related PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 108 (95% CI 55 to 180) (P=0.001)</td>
</tr>
<tr>
<td>2002</td>
<td>Bosentan 125 mg Bid v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>213 patients: IPAH, heritable, drug induced PAH, CTD-PAH (scleroderma and lupus)</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 44 (21 to 67) m (P=0.001)</td>
</tr>
<tr>
<td>2002</td>
<td>SC treprostinil v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>470 patients: IPAH, heritable, drug induced PAH, CTD-PAH, CHD-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 14.4 (4.4 to 27.6) m (P=0.006)</td>
</tr>
<tr>
<td>2005 (SUPER)</td>
<td>Sildenafil (20 mg, 40 mg, and 80 mg) v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>278 patients: IPAH, CTD-PAH, CHD-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups v placebo: 20 mg—45 (21 to 70) m (P=0.01); 40 mg—46 (20 to 72) m (P=0.01); 80 mg—50 (23 to 70) m (P=0.01)</td>
</tr>
<tr>
<td>2008 ARIES 1</td>
<td>Ambisentan (5 mg and 10 mg) v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>202 patients: IPAH, drug induced PAH, CTD-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups v placebo: 5 mg—31 (3 to 59) m (P=0.0008); 10 mg—51 (21 to 76) m (P=0.001)</td>
</tr>
<tr>
<td>2008 ARIES 2</td>
<td>Ambisentan (2.5 mg and 5 mg) v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>192 patients: IPAH, drug induced PAH, CTD-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups v placebo: 2.5 mg—32 (2 to 63) m (P=0.008); 5 mg—59 (30 to 89) m (P=0.001)</td>
</tr>
<tr>
<td>2009 (PHIRST)</td>
<td>Tadalafil (2.5 mg, 10 mg, 20 mg, and 40 mg) v placebo</td>
<td>16 week, prospective, randomized, double blind trial</td>
<td>605 patients: IPAH, drug induced PAH, CTD-PAH, CHD-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups v placebo: 2.5 mg—14 (6 to 33) m (P=NS); 10 mg—20 (1 to 39) m (P=NS); 20 mg—27 (11 to 44) m (P=NS); 40 mg—33 (15 to 50) m (P=0.01)</td>
</tr>
<tr>
<td>2010</td>
<td>Inhaled treprostinil 9 breaths QID v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>225 patients on background bosentan or sildenafil; IPAH, heritable, CTD-PAH, HIV-PAH, drug induced PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 20 (8 to 32.8) m (P=0.001)</td>
</tr>
<tr>
<td>2013 (PATENT)</td>
<td>Bosentan 25 mg v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>443 patients: IPAH, heritable, CTD-PAH, CHD-PAH, PpPH, drug induced PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 36 m (95% CI, 20 to 52 m, P&lt;0.001)</td>
</tr>
<tr>
<td>2012 (FREEDOM M)</td>
<td>Oral treprostinil v placebo</td>
<td>16 week, prospective, randomized, double blind trial</td>
<td>350 patients on ERA, PDE5 inhibitor, or both: IPAH, heritable, drug induced PAH, HIV-PAH, drug induced PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 11 (0 to 22) m (P=0.07)</td>
</tr>
<tr>
<td>2013 (FREEDOM C)</td>
<td>Oral treprostinil v placebo</td>
<td>12 week, prospective, randomized, double blind trial</td>
<td>349 treatment-naive patients: IPAH, heritable, drug induced PAH, CTD-PAH, CHD-PAH, HIV-PAH</td>
<td>6MWD</td>
<td>Difference in 6MWD between treatment groups: 23.0 (4 to 41) m (P=0.0125)</td>
</tr>
<tr>
<td>2013 (SERAPHIN)</td>
<td>Macitentan (3 mg and 10 mg) v placebo</td>
<td>Event driven, prospective, randomized, double blind trial</td>
<td>750 patients: IPAH, heritable, drug induced PAH, CTD-PAH, CHD-PAH, HIV-PAH</td>
<td>Time to composite endpoint of death, worsening of PAH, initiation of IV/SC prostanoids, lung transplant, atrial septostomy</td>
<td>HR: 3 mg dose—0.70 (95% CI 0.52 to 0.96) (P=0.01); 10 mg dose—0.55 (0.39 to 0.76) (P=0.001)</td>
</tr>
<tr>
<td>2015 (GRIPHON)</td>
<td>Selexipag v placebo</td>
<td>Event driven, prospective, randomized, double blind trial</td>
<td>1156 patients: IPAH, heritable, drug induced PAH, CTD-PAH, CHD-PAH, HIV-PAH</td>
<td>Time to composite endpoint of death, PAH hospital admission, initiation of IV/SC prostanoid therapy or long term oxygen therapy, lung transplantation, atrial septostomy</td>
<td>HR: 0.60 (99% CI 0.46 to 0.78) (P=0.001)</td>
</tr>
<tr>
<td>2015 (AMBITION)</td>
<td>Ambisentan + tadalafil v ambisentan or tadalafil monotherapy</td>
<td>Event driven, prospective, randomized, double blind trial</td>
<td>605 treatment-naive patients: IPAH, heritable, drug induced PAH, CTD-PAH, CHD-PAH, HIV-PAH</td>
<td>Time to composite endpoint of death, PAH hospital admission, PAH worsening, unsatisfactory long term clinical response</td>
<td>HR for combination therapy vs pooled monotherapy: 0.50 (95% CI 0.35 to 0.72) (P=0.001)</td>
</tr>
</tbody>
</table>

6MWD = six minute walk distance; BID = twice daily; CHD = congenital heart disease; CTD = connective tissue disease; ERA = endothelin receptor blockers; HR = hazard ratio; IV = intravenous; PAH = pulmonary arterial hypertension; PDE5 = phosphodiesterase; QID = four times a day; SC = subcutaneous.

in patients with mild symptoms (WHO functional class II). Compared with bosentan, macitentan has greater tissue penetration and produces more sustained receptor blockade. Macitentan 3 mg and 10 mg daily reduce a composite endpoint of long term morbidity and mortality by 30% and 45%, respectively (table 3). Importantly, this is driven exclusively by the reduction in worsening of PAH, and no reduction is seen in either all cause or PAH related mortality. Ambisentan (10 mg daily), a selective ERA antagonist, improves 6MWD, time to clinical worsening, WHO functional class, quality of life, and NT-proBNP when given as monotherapy. The major adverse effects of ERAs include hepatotoxicity, peripheral edema, anemia, and nasal congestion.

In a meta-analysis, hepatotoxicity was more commonly observed with bosentan, anemia with bosentan and macitentan, and peripheral edema with bosentan and ambisentan. Monthly liver function testing is warranted in patients taking bosentan. Although not mandated, serial liver function testing is advisable in patients taking macitentan and ambisentan. ERAs should be discontinued if liver aminotransferases are more than five times the upper limit of normal, if aminotransferase elevations are accompanied by an increase in serum bilirubin concentrations (more than twice the upper limit of normal), or if patients develop signs of hepatic failure.
Bosentan has several relevant drug interactions, notably decreasing serum sildenafil concentrations, which can impair the clinical benefits of combining these drugs.\textsuperscript{91, 92} Bosentan decreases warfarin concentrations, necessitating close monitoring of the international normalized ratio.\textsuperscript{93}

**Drugs targeting the prostacyclin pathway**

Prostacyclin and prostanooids bind IP receptors, which increases cyclic adenosine monophosphate concentrations causing non-selective pulmonary vasodilatation.\textsuperscript{94} They also have antiplatelet, antithrombotic, antiproliferative, and anti-inflammatory properties.\textsuperscript{95} Prostacyclin expression is reduced in the lungs of patients with PAH.\textsuperscript{7} Depending on the preparation and specific molecule, prostanooids can be given as a continuous infusion (intravenously (epoprostenol and treprostinil)) or subcutaneously ((treprostinil)), via inhalation (iloprost and treprostinil), or orally (treprostinil and beraprost).

Epoprostenol is a synthetic prostacyclin analog. It has a very short half life (less than five minutes) and is unstable at room temperature. Therefore, it must be refrigerated and administered by continuous intravenous infusion. Epoprostenol improves exercise capacity, mPAP, PVR, cardiac output, quality of life, and survival (table 3).\textsuperscript{74, 75} The administration of epoprostenol requires a complex delivery system including a continuous infusion pump, central venous catheter, and ice packs to maintain temperature. It must be administered through a central venous catheter, as it is an irritant to the peripheral veins. A newer formulation (Veletri) is stable at room temperature, simplifying administration. Epoprostenol is usually started at 2 ng/kg/min, and the dose is gradually increased. The average dose of epoprostenol used for PAH ranges from 25 to 40 ng/kg/min. The most frequent adverse effects include gastrointestinal symptoms (nausea, vomiting, and diarrhea), headache, flushing, and jaw pain.

Treprostinil is a prostacyclin analog that has several advantages, including a longer half life (three hours) and stability at room temperature. Various formulations of treprostinil are approved to treat PAH (subcutaneous, intravenous, inhalational, and oral). Subcutaneous treprostinil improves 6MWD, hemodynamics, and quality of life (table 3).\textsuperscript{95} The major limitation of subcutaneous administration is infusion site pain, which occurs in 85% of patients.\textsuperscript{95} Although intravenous treprostinil has not been studied in a randomized trial, the US Food and Drug Administration approved its use for PAH on the basis of bioequivalence. Open label, long term extension studies have shown improvement in exercise capacity and hemodynamics and delay in time to clinical worsening with intravenous treprostinil.\textsuperscript{96} Subcutaneous or intravenous treprostinil is started at 2 ng/kg/min and increased gradually to an average target dose of 60-80 ng/kg/min. The adverse effects of treprostinil and epoprostenol are similar.

Catheter related bloodstream infection in patients receiving intravenous prostanooids can be life threatening. Patients must apply a meticulously sterile technique to reduce infection when refilling the pump at home. A fully implantable pump decreases catheter related side effects and increases patients’ satisfaction;\textsuperscript{97} however, this pump is not approved for clinical use.

Inhaled treprostinil improves 6MWD and quality of life as an add-on therapy for patients who have symptoms despite taking sildenafil or bosentan (table 3).\textsuperscript{60} Inhaled treprostinil is started at three inhalations four times a day and increased gradually to a maximum of nine to 12 inhalations four times a day. It has minimal systemic effects owing to reduced systemic absorption and also lacks catheter related side effects. The common adverse effects include dry cough and headache.\textsuperscript{90}

Treprostinil diolamine is an oral, salt form of treprostinil. It improves 6MWD in treatment-naive PAH patients with no improvement in WHO functional class or time to clinical worsening.\textsuperscript{14, 92} However, no significant benefit is seen when oral treprostinil is used as an add-on therapy in patients who have symptoms when taking an ERA or a PDE5 inhibitor.\textsuperscript{85} Thus, oral treprostinil is approved only as a monotherapy for improving exercise capacity in treatment-naive PAH patients. Oral treprostinil is started at 0.125 mg three times a day and increased by 0.125 mg every three to four days. Common adverse effects include nausea, diarrhea, headache, and jaw pain.\textsuperscript{82}

Selexipag is an orally available, non-prostanoid activator of IP receptors.\textsuperscript{80} Both the parent drug and its metabolite have a high affinity for IP receptors. This selectivity minimizes adverse effects and facilitates dose escalation. In a long term, event driven trial, selexipag reduced the composite endpoint of death, lung transplantation, atrial septostomy, hospital admission for worsening PAH, or worsening of PAH by 40% (table 3).\textsuperscript{61} The benefits were mainly driven by reduction in hospital admissions for PAH, with no improvement in mortality. The reduction in morbidity was not dose dependent. Selexipag is started at 200 µg twice daily and increased weekly to a maximum of 1600 µg twice daily. The common adverse effects include nausea, vomiting, diarrhea, headache, and jaw pain.

**Treatment approach**

Patients with positive acute vasodilator testing should be treated initially with CCBs and monitored closely. Non-responders to vasodilator are stratified on the basis of clinical, echocardiographic, and hemodynamic evaluations that assess right ventricular function as high or low risk (table 4). Patients with a high risk profile have worse survival.\textsuperscript{99-101} Thus, patients at high risk should be considered for initial parenteral prostanooid therapy. Epoprostenol is preferred in these patients given its survival benefit. In a retrospective review of 19 patients at high risk presenting with cardiac index less than 2 L/min/m\textsuperscript{2} and PVR greater than 20 WU, starting triple combination therapy with epoprostenol, a PDE5 inhibitor, and an ERA at diagnosis was associated with improvement in exercise capacity and hemodynamics and 100% survival at three years.\textsuperscript{102} The efficacy, safety, and cost effectiveness of this approach needs further assessment in a randomized controlled trial.

Patients with a low risk profile are treated by either sequential combination therapy or initial combination therapy. In the first approach, patients are started on oral monotherapy. A second drug, targeting a different
**Pathogenesis of PAH**

Despite the benefits of current therapies to the quality of life and time to clinical worsening, these treatments do not decrease mortality, apart from epoprostenol which improved survival in PAH patients with WHO functional class IV. The average improvement in functional capacity (6MWD) and hemodynamics (mPAP) for each of the four classes of PAH specific therapies is modest. In randomized clinical trials, the average fall in mPAP with an optimal dose of sGC, PDE5 inhibitor, ERA, or prostanoid is less than 6 mm Hg (table 5). The primary criticism of current classes of PAH specific therapies, beyond their limited hemodynamic efficacy, is that they primarily target excessive vasoconstriction, which is a dominant pathophysiologic feature in less than 10% of patients with PAH. Better understanding of disease pathogenesis is thus needed to identify new targets for therapy. Ongoing research has provided new understanding of the cellular, genetic, and epigenetic changes that drive pathological remodeling in the lungs of patients with PAH (fig 7). Box 2 describes the five emerging concepts in the pathogenesis of PAH.

**Cytosolic calcium and ion channels**

Increased cytosolic calcium ([Ca\(^{2+}\)]\(_{cyt}\)) contributes to the contractile, hyperproliferative, and anti-apoptotic phenotype of PAH PASMCs. [Ca\(^{2+}\)]\(_{cyt}\) is regulated by several ion channels that control calcium influx, as well as Cyt\(^{2+}\) sequestration within the sarcoplasmic reticulum and mitochondria. Elevated [Ca\(^{2+}\)]\(_{cyt}\) in PASMCs from patients with PAH has been linked to the activation of store operated Ca\(^{2+}\) channels, including the transient receptor potential channel TrpC6, and downregulation of voltage gated potassium channels, such as Kv1.5. Decreased expression of Kv1.5 channels, which normally maintain PASMC membrane potential, leads to membrane depolarization and influx of Ca\(^{2+}\) through voltage dependent calcium channels (Ca\(_{\text{L}}\)). In rodent models of candidates for transplant, Veno-arterial extracorporeal membrane oxygenators and pumpless oxygenators (Novo lung) have also been used successfully as a bridge to transplant in patients with PAH and RVF.103

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**Fig 7** | Mechanisms implicated in pathogenesis of pulmonary arterial hypertension (PAH). PAH is a panvasculopathy, meaning that all layers of the vascular wall are involved. PAH is also reflective of gene environment interactions and has important genetic and epigenetic mechanisms. This figure shows abnormalities in the gene and environment, blood, and each layer of the pulmonary artery, from intima (endothelial cells), to media (pulmonary arterial smooth muscle cells—PASMCs) to adventitia (fibroblasts). Because of the many reports that inform this composite figure, individual sources for the information are not referenced. The normal state is shown on the left side, the abnormalities that occur in PAH are highlighted in the middle section, and the consequences of these abnormalities are shown on the right. The net effect of these abnormalities is a state of vasoconstriction, inflammation, thrombosis with a hyperproliferative, apoptosis resistant PASMC population, which promotes vasoconstriction and vascular obstruction, and excessive fibrosis, which reduces vascular compliance. These vascular changes ultimately increase right ventricular (RV) afterload and impair RV-pulmonary artery coupling, leading to RV failure. 5-HHT=5 hydroxytryptamine; ADMA=asymmetric dimethylarginine; APN=adiponectin; BMPR2=bone morphogenetic protein receptor 2; BNP=brain natriuretic peptide; Ca=calcium; DNAMT=DNA methyltransferase; Drp-1=dynamin related protein 1; ET1=endothelin; HDAC=histone deacetylases; HIF=hypoxia inducible factor; IL=interleukin; MCP-1=monocyte chemoattractant protein-1; MCUC=mitochondrial calcium uniporter complex; miRNA=miRNA; MMP=matrix metalloproteinase; NFAT=nuclear factor of activated T cells; NF-kB=nuclear factor kappa light chain enhancer of activated B cells; NO=nitric oxide; PDGFR=platelet derived growth factor receptor; PDGR=platelet derived growth factor receptor; PDH=pyruvate dehydrogenase; PDK=pyruvate dehydrogenase kinase; PGI2=prostacyclin; PKM-2=pyruvate kinase M2; PPAR=peroxisome proliferator activated receptor; SERCA=sarco-endoplasmic reticulum Ca ATPase; SERT=serotonin transporter; SNP=single nucleotide polymorphism; SOD=superoxide dismutase; Th2=T helper cells; TNF=tumor necrosis factor; TRPC=transient receptor potential cation channel; T-reg=regulatory T cells; TXA2=thromboxane A2; VIP=vasoactive intestinal peptide

pulmonary hypertension, restoring Kv1.5 expression through adenosinergic gene transfer improves hemodynamics.\(^1\)\(^1\) In addition to promoting smooth muscle cell contractility, prolonged increases in \([\text{Ca}^{2+}]_i\) can increase contractility by driving cells into the cell cycle.\(^1\)\(^9\) Activation of the Ca\(^{2+}\)/calcineurin sensitive transcription factor nuclear factor of activated T cells (NFAT) in PAH PASMCs perpetuates the elevation in \([\text{Ca}^{2+}]_i\) by suppressing Kv1.5 expression.\(^1\)\(^2\) NFAT activation also promotes apoptosis resistance by increasing expression of bcl-2.\(^1\)\(^2\) Failure
Mitochondrial metabolic dysfunction

Mitochondrial dysfunction in PAH PASMCs includes a metabolic shift from glucose oxidation toward uncoupled aerobic glycolysis, a metabolic pattern first described by Otto Warburg in cancer cells. In the presence of oxygen, rates of glycolysis in normal vascular cells are closely coupled to rates of glucose oxidation. In Warburg metabolism, uncoupled glycolysis is increased because, whereas mitochondrial respiration (glucose oxidation) is actively suppressed, glycolysis is disproportionately elevated providing the abnormal cell with sufficient energy to thrive. Although this disease signature in PAH was initially identified in PASMCS, similar metabolic and mitochondrial changes occur in pulmonary artery endothelial cells (PAECs) and adventitial fibroblasts of PAH patients. More recently, examination of the diseased right ventricle in PAH patients and animal models has identified a similar Warburg phenotype in cardiomyocytes, where it reduces contractility.

In healthy cells, the pyruvate produced by glycolysis enters the mitochondria via the mitochondrial pyruvate transporter. There it is converted by pyruvate dehydrogenase (PDH) into acetyl coenzyme A, which fuels the Krebs cycle. In PAH, oxidative phosphorylation is actively suppressed by upregulated expression of pyruvate dehydrogenase kinase (PDK). PDK phosphorylates and inhibits PDH. This shifts the cell to rely on glycolysis, which is energetically inefficient. However, in PAH, glycolysis is upregulated, both by an increase in glucose influx mediated by increased glucose transporter (glut) expression and by alterations in splice variant expression of the terminal glycolytic enzyme pyruvate kinase. This metabolic shift supports rapid proliferation while avoiding mitochondrial apoptosis.

Warburg metabolism accounts for the increase in fluorodeoxyglucose uptake in the lungs and right ventricle of PAH patients and preclinical PAH models observed using positron emission tomography. In patients with PAH, a lactate gradient exists from superior vena cava to pulmonary artery. Whether this pre-pulmonary lactate gradient reflects right ventricular ischemia or Warburg metabolism is unknown.

Emerging metabolic therapies that exploit this pathway include the small molecule PDK inhibitor dichloroacetate. Dichloroacetate reverses the Warburg phenotype in PASMCs and right ventricular cardiomyocytes and regresses PAH in preclinical models. Dichloroacetate has been safely used to chronically treat children with mitochondrial disease and lactic acidoses. A four month, open label study of dichloroacetate (3 to 6.25 mg/kg twice daily) in patients with IPAH taking approved PAH therapies showed that dichloroacetate reduced mPAP and PVR while improving 6MWT; however, some patients did not respond to dichloroacetate, and these patients had functional variants of SIRT3 and UCP2.

Vascular cells isolated from patients with PAH also show additional signs of mitochondrial dysfunction, including fragmentation and membrane hyperpolarization. Hyperpolarization of mitochondrial membranes contributes to apoptosis resistance by blocking the release of pro-apoptotic mediators, such as cytochrome C.(103). The mitochondria in vascular cells exist in dynamic networks that are continuously dividing (fission) and joining together (fusion). For nuclear division to occur, mitosis must be coordinated with mitochondrial division. Increased rates of mitotic fission in PAH cause PAH PASMCs to show fragmented mitochondria (fig 8), and this can be therapeutically targeted. A fission/fusion imbalance in PAH results from increased activation of the mitochondrial fission mediator dynamin related protein 1 (Drp1) and reduced expression of the fusion mediator, mitofusin 2. Drp1 activation and mitofusin 2 downregulation are secondary to other changes in
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Genetic contributions to PAH

Heritable forms of PAH account for 6-10% of all PAH.
Homozygous, germline mutations in BMPR2, the gene encoding the type II bone morphogenetic protein (BMP) receptor (BMPR-II), account for 70-80% of heritable PAH cases, as well as 15-25% of IPAH cases.

Box 3 describes the discovery of BMPR2 gene mutations in PAH. At risk siblings from families with PAH carrying a BMPR2 mutation have a disease penetrance in mutation carriers of only 27%. Disease penetrance is greater in females (42%) than in males (14%). Thus, a BMPR2 mutation increases an individual’s chance of developing PAH 25,000-fold, from roughly 1 in 100,000 to 1 in 4.5

A meta-analysis of PAH patients also showed that, compared with patients without BMPR2 mutations, mutation carriers are younger at diagnosis, develop more hemodynamically severe disease, are less likely to respond to vasodilators, and are at an increased risk of death.

Fig 8 | Mitochondrial fragmentation in pulmonary arterial hypertension (PAH). Confocal imaging of mitochondria in human pulmonary arterial smooth muscle cells (PASMCs). Left side: Mitochondria are stained red with potentiometric dye tetramethylrhodamine methyl ester. Nuclei are blue (stained with 6-diamidino-2-phenylindole). Note fused network in normal mitochondria versus fragmented network in PAH PASMC. This fragmentation reflects increase in mitotic fission in PAH that results from increased expression of activated dynamin related protein 1 and reduced expression of fission mediator mitofusin 2. Right side: To directly measure fission, PASMC were transfected with mitochondrial matrix targeted, photoactivatable green fluorescent protein (mito-PA-GFP) and mitochondrial targeted red fluorescent protein (mito-Ds-red). Mito-Ds-red is tonically fluorescent whereas mito-PA-GFP does not fluoresce until photoactivated. See supplemental movies for dynamic images of these files. In these movies, mito-PA-GFP is selectively activated in a few mitochondria (using a focused 488 nm laser) and serial observations allow measurement of spread of green protein within adjoined mitochondria. More fissioned network in PAH PASMC has less spread of matrix GFP green signal outside activation zone (white box) than does control PASMC imaged at same time interval.

Reduced expression of the MCU in PASMCs from PAH patients and rodent models provides a potential unifying mechanism linking dysregulated mitochondrial metabolism and dynamics and partially explains the observed increase in PASMC proliferation and apoptosis resistance in PAH. MCU is the major functional subunit of the MCU complex that allows for the influx of Ca^{2+} into the mitochondrial matrix. Reduced expression of MCU (and increased expression of MICU1, a negative regulator of MCU) increases [Ca^{2+}]_{mito} in PAH while lowering intramitochondrial calcium. Increased [Ca^{2+}]_{mito} promotes vasoconstriction and enhances mitochondrial fission and proliferation. Low intramitochondrial calcium inhibits calcium sensitive dehydrogenases in the mitochondrial matrix (including PDH) and thereby inhibits glucose oxidation. MCU downregulation in PAH is epigenetically mediated by increases in the micro-RNAs miR25 and miR138. Anti-miRs or MCU gene transfer reverse the mitochondrial phenotype in PAH PASMCs and regress PAH in the monocrotaline model.
However, BMPR2 mutations are considered to be permissive of disease, requiring additional genetic, epigenetic, or environmental factors for the development of PAH in people with mutations.

BMPR-II protein concentrations are reduced by about 75% in lung tissue and endothelial cells from patients with PAH.\(^{140} 142\) This reduction is greater than expected from haploinsufficiency alone and occurs in patients lacking BMPR2 mutations, as well as in non-genetic rodent models.\(^{140} 141\) This suggests that factors associated with disease, independent of mutation status, suppress BMPR-II expression and provide biologic plausibility that targeting BMPR-II deficiency might be beneficial even in PAH patients lacking BMPR2 mutations.

Impaired BMPR-II signaling has been shown to promote accelerated cell proliferation,\(^{152}\) while potentially contributing to disease initiation by enhancing the susceptibility of PAECs to apoptosis.\(^{153}\) Loss of BMPR2 in the endothelium also causes mitochondrial dysfunction and inflammation,\(^{115}\) providing a potential mechanism linking BMPR2 mutations to mitochondrial dysfunction in PAH. Emerging BMPR-II related therapies for PAH include strategies to rescue the functionality of mutated BMPR2 alleles or enhance BMPR-II signaling through the functional receptors that are produced by the non-mutated allele. Rescue strategies include the use of read through compounds such as Ataluren (PTC-124), which promote the transcriptional read through of premature termination codons and the production of functional, full length BMPR-II protein from mutated alleles.\(^{144}\) Missense mutations can also be rescued in preclinical studies by using chemical chaperones, such as 4-phenylbutyrate, which increase trafficking of misfolded, but otherwise functional, BMPR-II protein from the endoplasmic reticulum to the cell surface.\(^{145}\) Preclinical trials of lung targeted BMPR-II gene therapy have had mixed results.\(^{146} 147\) Examples of enhancing signaling via non-mutated BMPR-II protein include the delivery of recombinant BMP ligands, such as BMP9,\(^{143}\) or small molecule agonists of canonical BMP signaling, such as FK506.\(^{148}\) These approaches enhance BMPR-II mediated signaling in the endothelium and can reverse established disease in the Sugen 5416/Eng/Eng hypoxia rat model.\(^{143} 148\) Inhibition of BMPR-II degradation, through lysosomal inhibitors such as hydroxychloroquine, also enhances BMPR-II mediated signaling in human PAECs and prevents disease in the monocrotaline model.\(^{149}\)

Mutations in other components of the BMP signaling pathway including ACVR1I, which encodes the type I BMP receptor ALK1, ENG, the gene encoding the accessory receptor endoglin, and SMAD9, which encodes the BMP transcriptional mediator, Smad 8, contribute to a small percentage of PAH cases.\(^{150} 155\) Whole exome sequencing studies of families with PAH have also identified mutations in genes unrelated to canonical BMP signaling pathways in 1-3% of all cases of PAH. These include mutations in KCNK3, which encodes the pH sensitive potassium channel TASK-1,\(^{156} 157\) and CAV1, which encodes caveolin 1, a membrane protein that is essential for the formation of lipid rafts, known as caveolae.\(^{155}\) Whole exome sequencing approaches have also been used in the examination of pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH), which are classified as group 1 pulmonary hypertension. Independent studies examining families with autosomal recessive forms of either PVOD or PCH identified bi-allelic mutations of EIF2AK4 (or GCN2), the gene encoding eukaryotic translation initiation factor 2 kinase, as the mutation underlying the hereditary forms of these conditions.\(^{156} 157\) Thus, genotyping shows that PVOD and PCH are phenotypic variants of the same disease. Bi-allelic EIF2AK4 mutations were also identified in 20-25% of sporadic cases of both conditions.\(^{154} 158\)

Preliminary data from the PAH Biobank sponsored by the National Heart, Lung, and Blood Institute at Cincinnati Children’s Hospital Medical Center have been provided by study principal investigator William Nichols (CCHMC/University of Cincinnati). The incidence of pathogenic/suspected pathogenic gene variants identified, using panel sequencing of 12 genes in the 2251 PAH patients, is 10.8%. Pathogenic/suspected pathogenic variants are less frequent in APAH (5.8%) and more common in IPAH (13.2%) (personal communication, W Nichols). Nicholas Morrell (University of Cambridge) is conducting a study of IPAH and heritable PAH patients (n=1250), using whole genome sequencing as part of the National Institutes for Health Research Bioresource for Rare Diseases Study. These two studies will ultimately define the genetic contribution to various PAH patient populations and help to determine the value of routine genetic testing in patients with PAH.

**Involvement of epigenetic factors in PAH**

The expression of genes in PAH is also influenced through epigenetic processes, defined as mechanisms that alter gene expression without changing the sequence of genomic DNA. Epigenetic mechanisms in PAH include DNA methylation, histone modification, and RNA interference via micro-RNAs (box 4).

**DNA methylation**

Epigenetic regulation of a pulmonary hypertension phenotype was first identified in the assessment of spontaneous PAH in the fawn hooded rat model.\(^{159}\) Hypermethylation and silencing of the gene encoding superoxide dismutase 2 in this model caused a 50% reduction in pulmonary artery superoxide dismutase 2 protein expression (also seen in PAH patients). Superoxide dismutase 2 is a mitochondrial enzyme that converts superoxide to hydrogen peroxide. Hydrogen peroxide serves as a diffusible redox signaling molecule.

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**Box 3: Discovery of BMPR2 mutations in PAH**

1997—First description of “primary pulmonary hypertension” by Dresdale includes recognition of heritable PAH

1997—Microsatellite markers and linkage analysis are used to map the “PAH gene” to a region on chromosome 2q31-32

2000—Two independent groups identify the affected gene as BMPR2, a receptor of the transforming growth factor-β superfamily.\(^{150} 151\)

1951—First description of “primary pulmonary hypertension” by Dresdale includes recognition of heritable PAH

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2000—Two independent groups identify the affected gene as BMPR2, a receptor of the transforming growth factor-β superfamily.\(^{150} 151\)
Reduced hydrogen peroxide in the fawn hooded rat model causes normoxic activation of hypoxia inducible factor (HIF-1α), which triggers the Warburg effect by upregulating PDK and glut expression and increased PASMC proliferation, and also inhibits Kv1.5 channel function/expression, causing vasoconstriction. This epigenetic mechanism results from lung specific increases in the expression of DNA methyltransferases 1 and 3B (DNMT). A similar mechanism of reduced superoxide dismutase 2 and elevated HIF-1α occurs in endothelial cells from patients with IPAH. Increased DNA methylation is implicated in the exaggerated pulmonary hypertensive response of mice born to mothers subjected to a restrictive diet during pregnancy. Future therapies may involve inhibiting DNA methyltransferases, as is currently done in some hematologic malignancies (using decitabine), modulating the enzymes that demethylate DNA (ten eleven dioxygenases), and/or manipulating methyl binding proteins, which alter the transcription of methylated genes.

**Histone modification**

An examination of lung tissue from patients with IPAH, as well as lungs and right ventricles from rats with chronic hypoxic pulmonary hypertension, identified increased expression of histone deacetylases (HDAC1 and HDAC5) in remodeled pulmonary vessels. Additional work in PAECs from PAH patients identified increased nuclear accumulation of HDAC4 and HDAC5, leading to down-regulation of the transcription factor myocyte enhancer factor 2 and a reduction of several genes involved in pulmonary vascular integrity and homeostasis, including Krüppel-like factors 2 and 4 and connexins 37 and 40. Interestingly some metabolic enzymes, such as PDH, act within the nucleus to generate the acetyl coenzyme A required for histone regulation. Increased histone acetylation has also been reported in a rat model of persistent pulmonary hypertension in the newborn, where a compensatory sixfold increase in eNOS expression was attributed to enhanced acetylation of the eNOS promoter and a modest decrease in gene methylation.

The HDAC inhibitors valproic acid and suberoylanilide hydroxamic acid reduced established hypoxia induced pulmonary hypertension in rodent models. Similar results were also obtained with the HDAC inhibitor MGCD0103, which prevented hypoxic pulmonary vascular remodeling in rats. Although valproic acid improves RVH in both the monocrotaline rat model and the pulmonary artery banding model of RVF, another HDAC inhibitor, trichostatin A, worsened RVH and fibrosis in the rat pulmonary artery banding model. These differences may reflect the need to target specific subtypes of HDAC in PAH and/or differences in the role of HDACs in various forms of RVH.

**Micro-RNAs**

Micro-RNA dysregulation occurs in whole lung tissue in PAH, as well as in cultured PAECs, PASMCs, and fibroblasts. Table 6 summarizes the miRs identified in PAH and their effect on in vitro or in vivo processes. Many of these dysregulated miRs influence pathways that are critical to creating the cancer-like, mitochondrial-metabolic phenotype of PAH. Therapeutic modulation of disease associated miRs, using miR-mimics or antago-miRs, has shown potential to prevent or reverse PAH in rodent models. Although these findings are promising, the ability of miRs to regulate a broad array of genes, which is the very basis of their value, also raises the potential for undesirable off-target effects that could limit their translation into the treatment of human disease. miRs, particularly those present at altered concentrations in the circulation, represent useful biomarkers that can predict survival.

Two recent studies provide an example of the intersection of genetics, epigenetics, and metabolism in the pathogenesis of PAH. Caruso et al and Zhang et al showed that altered activity of the enzyme pyruvate kinase contributes to the Warburg phenomenon in endothelial cells and fibroblasts in PAH, respectively. Pyruvate kinase is the final step in glycolysis, catalyzing phosphate transfer from phosphoenolpyruvate to ADP, producing pyruvate and ATP. Both groups showed that this pro-glycolytic mechanism is stimulated by upregulation of a heterogeneous nuclear ribonucleoprotein, polyribimidine tract binding protein (PTBP1), in response to an epigenetic mechanism—namely, downregulation of miR124. The increase in PTBP1 changes the splice variant expression of pyruvate kinase, favoring the proglycolytic variant pyruvate kinase M2. The mitochondrial supply of pyruvate is further compromised by downregulation of the mitochondrial pyruvate transporter. Remarkably, BMPR2 mutation/downregulation leads to the same Warburg phenotype and causes proliferation of endothelial cells. This work shows coherence between apparently disparate theories, illustrating how seemingly unrelated gene mutation or epigenetic changes can promote the Warburg mitochondrial metabolic phenotype, a final common pathway of the proliferative PAH phenotype in many vascular cells.
Inflammation and fibrosis in PAH

Chronic inflammation and maladaptive fibrosis play an essential role in PAH. Immune dysfunction is a central feature of APAH, especially in patients with connective tissue diseases (for example, systemic lupus erythematosus and scleroderma) and infections (for example, HIV and schistosomiasis). The prevalence of schistosomiasis in the developing world makes it the single largest cause of PAH, with an estimated 2,000,000 cases worldwide. Chronic inflammation is also observed in IPAH. Histologic examinations of lung tissue from IPAH patients have identified the presence of immune cell infiltrates, composed of lymphocytes, macrophages, dendritic cells, and mast cells in pulmonary vascular lesions. Circulating concentrations of inflammatory cytokines including interleukins 1β, 2, 4, 6, 8, 10, and 12p70, tumor necrosis factor α, and the chemokines CX3CL1 (or fractalkine), CCL2 (or monocyte chemotactic protein 1), and CCL5 (or RANTES) are elevated in IPAH. Elevated concentrations of interleukins 6, 8, 10, and 12 predicted survival in a cohort of 21 PAH patients better than traditional prognostic markers, such as 6MWD or cardiopulmonary hemodynamics.

Fibroblasts isolated from the adventitia of pulmonary arteries from both PAH patients and chronic hypoxia models of pulmonary hypertension show a hyperproliferative, apoptosis resistant, pro-inflammatory phenotype, marked by the production of inflammatory molecules and expression of myofibroblast markers. These changes are associated with epigenetic modifications including increased HDAC activity and decreased miR124 expression. Interestingly, the depletion of circulating mononuclear cell populations blocks adventitial remodeling in a rat model of hypoxic pulmonary hypertension, suggesting that fibrotic remodeling is at least partially dependent on the recruitment of circulating mononuclear mesenchymal precursors. The interplay between adventitial fibroblasts and monocyctic cells is further supported by the demonstration that human and experimental PAH fibroblasts activate a STAT3 mediated, pro-inflammatory phenotype in macrophages by means of increased interleukin 6 secretion.

Recent mechanistic studies examining the contribution of cellular immunity to the pathogenesis of pulmonary hypertension have also focused on the role of specific lymphocyte populations, including T cell, B cell, and natural killer cell subsets. The role of T lymphocytes in disease pathogenesis is controversial. Athymic nude rats lack mature T cells and develop more severe pulmonary hypertension in response to either monocrotaline or the vascular endothelial growth factor receptor blocker SU-5416, suggesting a protective role for regulatory T cells. In Sugen rats, PAH is attenuated by blockade of leukotriene B4 activity with leukotriene A4 hydrolase inhibitors. More recently, blockade of interleukin 6, using the anti-interleukin 6 receptor antibody MR16-1, was shown to prevent chronic hypoxia induced pulmonary hypertension in mice by blocking the accumulation of Th17 cell derived interleukin 21 and M2 macrophages in the lungs of these mice. Thus the role of T cell subsets in disease is context specific and likely varies among species.

The contribution of humoral immunity and excessive B cell activation to the pathogenesis of PAH is supported by multiple studies identifying circulating autoantibodies and the ectopic expansion of pulmonary lymphoid tissues in patients with PAH. Increased bronchus associated lymphoid tissues and autoantibody production has also been reported in the monocrotaline rat model, with passive autoantibody transfer being sufficient to transmit disease between animals.

Impairment of innate lymphocytes, such as natural killer cells, is reported in human and experimental models of PAH. For example, decreased expression of Fas, an important mediator of apoptosis, has been associated with decreased survival in PAH patients. Moreover, innate lymphocytes, such as natural killer cells, play a crucial role in the pathogenesis of PAH, with decreased expression of Fas, an important mediator of apoptosis, associated with decreased survival in PAH patients. Thus, the contribution of both adaptive and innate immunity to the pathogenesis of PAH is critical to the development of effective therapeutic strategies.
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Fig 9 | Right ventricular changes in pulmonary arterial hypertension (PAH). (A) Adaptive versus maladaptive right ventricular hypertrophy (RVH). Adaptive RVH on left is concentric, and patient had long survival with group 1 pulmonary hypertension before succumbing to cancer. Maladaptive patient (on right) had associated PAH (scleroderma) and died within three years of diagnosis with eccentric dilatation and thinning of right ventricle and right ventricular failure. Reproduced with permission from Rich S, et al. Chest 2010;138:1234-9. (B) Cardiac magnetic resonance imaging (MRI) showing severe dilatation right ventricle (RV) with flattening of interventricular septum and small left ventricle (LV). Reproduced with permission from Michelakis E, et al. Circulation 2003;108:2066-9. (C) Cardiac positron emission tomography (PET) scan showing increased fluorodeoxyglucose (FDG) uptake in right ventricle in patient with PAH. Reproduced with permission from Oikawa M, et al. JACC 2005;45:1849. (D) Cardiac MRI with gadolinium showing late gadolinium enhancement (white areas identified by arrows) in interventricular septum at right ventricular free wall insertion sites. LGE=late gadolinium enhancement

PAH. Natural killer cells can adopt an angiogenic phenotype and contribute to vascular remodeling in pregnancy and cancer through the production of angiogenic cytokines and matrix degrading enzymes. In PAH, natural killer cells have an impaired phenotype, marked by increased transforming growth factor β and matrix metalloproteinase 9 production. Deficiencies in natural killer cells and CD8+ killer T cells were linked to an increased risk of death in a small cohort of IPAH and APAH patients.

Right ventricular dysfunction in PAH
The right ventricle’s ability to adapt to pressure overload determines functional status and prognosis in PAH. A dilated right ventricle or a small left ventricle independently predicts poor survival in PAH. Adult PAH patients with RVF admitted for inotropic therapy have a 41% acute mortality rate, far exceeding the mortality of patients admitted with left ventricular failure.

Chronic pressure overload in PAH stimulates RVH in a compensatory attempt to maintain cardiac output while minimizing wall stress (fig 9A, right panel). However, in some patients, the right ventricle dilates owing to maladaptive remodeling and eventually fails (fig 9A, left panel, and fig 9B). In general, PAH patients with congenital heart disease (Eisenmenger’s syndrome) show adaptive, concentric RVH and are free from RVF for decades; conversely, APAH patients, particularly those with scleroderma, have maladaptive remodeling with worse right ventricular function and a higher incidence and severity of RVF, despite lower mPAP.

Abnormalities of right ventricular perfusion, angiogenesis, autonomic signaling, metabolism, and fibrosis contribute to the development of maladaptive RVH and RVF. Many of the changes in the right ventricle during RVH, such as activation of the autonomic nervous system and uncoupled glycolysis, are ultimately maladaptive. Whether these chamber specific, cardiac abnormalities reflect primary insults to the right ventricle or are simply the maladaptive responses to increased right ventricular...
afterload remains uncertain. Nonetheless, they represent important pathophysiologic abnormalities and offer many therapeutic targets.

Right ventricular ischemia
Patients with PAH often present with evidence of myocardial ischemia, including angina-like chest pain and elevated concentrations of serum troponin. Right ventricular ischemia may reflect reduced right coronary artery perfusion pressure. However, right ventricular function is usually preserved as long as coronary perfusion pressure, the difference in pressure between the aorta and the right ventricle in systole or diastole, exceeds 50 mm Hg. In PAH, systemic (aortic) hypotension and a rise in right ventricular pressure (in both systole and diastole) combine to reduce the pressure gradient that drives perfusion of the right coronary artery, contributing to right ventricular ischemia. Ischemia in RVH may also reflect loss of small vessels (microvascular rarefaction). The observation that RVF is more prevalent in patients with scleroderma, who have circulating autoantibodies toxic to the endothelium, and in PAH models that are characterized by endothelial injury (induced by SU5416) suggests a potential role for primary endothelial damage in the right ventricular microcirculation. Capillary rarefaction in experimental PAH is reversible with β-adrenergic blockers.

Autonomic activation and downregulation of β1 receptors in RVH
The right ventricle is less responsive to inotropes than the left ventricle. Circulating catecholamines are elevated in PAH patients with RVF. The autonomic activation exceeds that seen in left ventricular failure. Like PAH patients, rats with PAH induced maladaptive RVH show selective downregulation and uncoupling of β1-adrenoreceptors, α1-adrenoreceptors, and dopaminergic receptors in right ventricular myocytes. This is due to G protein coupled receptor kinase 2 (GRK2) mediated uncoupling of receptor signaling and impairs responses to inotropes. GRK2 inhibition seems to be therapeutically beneficial in PAH. In rodent models of PAH and RVH, dobutamine is the optimal right ventricular inotropic; however, this has not been established in PAH patients. The observed downregulation of the cardiomyocyte β1-receptors suggests biologic plausibility for using β-adrenergic blockers for RVF in PAH patients. However, in a study of 19 patients, bisoprolol reduced cardiac output and exercise capacity with no improvement in RVEF. In contrast, carvedilol reduced glycolytic rate and increased β-adrenergic receptor expression in the right ventricle with improvement in right ventricular fractional area change and no reduction in exercise capacity and cardiac output. More definitive studies are needed.

Right ventricular metabolism in RVH
The fetal right ventricle relies on glycolysis and glucose oxidation as the major sources of ATP production. In the adult right ventricle, there is a transition to reliance on fatty acid oxidation (FAO), which accounts for 60-90% of ATP production. FAO needs about 12% more oxygen per mole of ATP than does glucose oxidation. The hypertrophied right ventricle depends on glucose metabolism. The metabolic fate of glucose in RVH is altered because processes, including PDK mediated inhibition of PDH, suppress mitochondrial metabolism. In this scenario, lactate (the end product of glycolysis) accumulates, causing acidosis and exacerbating right ventricular hypokinesis. Activating glucose oxidation by using the PDK inhibitor dichloroacetate improves right ventricular function while regressing RVH. Thus, the right ventricle in PAH can be resuscitated metabolically. Consistent with this, increased right ventricular uptake of fluorodeoxyglucose was observed in a small series of PAH patients studied with positron emission tomography, a consequence of reliance on uncoupled glycolysis, and was reduced by effective PAH targeted therapy (fig 9C).

In PAH patients, there is evidence of cardiac steatosis and lipotoxicity, which murine data suggest relates to reduced FAO. Whether differences in FAO occur in adaptive versus maladaptive RVH remains to be determined. In light of this report of impaired FAO in the right ventricles of patients with PAH, it is uncertain whether FAO inhibitors, such as trimetazidine and ranolazine, would be helpful in PAH, as they are in preclinical models of RVH induced by pulmonary artery banding.

Right ventricular hibernation
Right ventricular ischemia leads to right ventricular hibernation, defined as a reduction in right ventricular function caused by a chronic decrease in perfusion with persistent myocardial viability. Thus, future therapeutic opportunities might include anti-ischemic or angio-genic therapy. Alternatively, it might be empirically easier to treat RVF and ischemia by pharmacologically changing metabolism or adrenergic signaling to “do more with less” (that is, use of drugs that alter cardiac metabolism or anti-adrenergic drugs). However, the clinical trial data that would be needed to alter practice are lacking.

Right ventricular fibrosis
The role of right ventricular fibrosis in RVF in PAH is unknown. However, late gadolinium enhancement on MRI at right ventricular insertion points may indicate localized fibrosis (fig 9D) and is an independent risk factor for poor prognosis.

Chamber specific responses to RVH
Quantitative differences in the expression of many transporters and pumps in the right versus left ventricle, seen in fetal hearts, persist into adulthood. This raises the possibility that chamber selective therapeutic targets may exist in RVH. For example, PDE5 expression, normally absent in the right ventricle, is induced during RVH. Sildenafil has a direct right ventricular inotropic effect that occurs only in RVH.

Sex differences in PAH
Although woman are more likely to acquire PAH, men (particularly those aged over 60 years) have a worse prognosis. This may relate to more maladaptive response of the male right ventricle to PAH. Male patients are
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QUESTIONS FOR FUTURE RESEARCH

Clinical

• How should right ventricular function best be measured in PAH patients?
  – MRI versus three dimensional echocardiography versus two dimensional echocardiography
• What is the role of right ventricle relevant biomarkers?
• Is right ventricle-pulmonary artery coupling an important metric in PAH?
  – Can right ventricle-pulmonary artery coupling be modified therapeutically using drugs or mechanical devices?
• What is the role of assessing right ventricular contractile reserve in patients with PAH?
• Should genetic testing be routinely done in patients with confirmed PAH and their family members?
• Screening for associated PAH
  – How often should patients with risk factors for PAH (eg, scleroderma or HIV infection) have screening echocardiograms for diagnosing PAH?
• Is the initial combination PAH targeted therapy strategy superior to a sequential combination therapy strategy?
  – What is the health economic implication of initial combination therapy?
• The use of inotropes in PAH patients with RVF is associated with high mortality rates, and the inotrope of preference varies tremendously between and within centers.
  – What is the optimal right ventricular inotrope for use in PAH patients?
  – Are inotropes increasing mortality in PAH?
• Is there a role for right ventricular assist devices or oxygenators in supporting PAH patients?
• Therapeutic application of drugs approved for group 1 pulmonary hypertension in other groups (with the exception of riociguat for group 4 disease) is not supported by evidence
  – How can therapeutic creep best be avoided?
  – Are there subsets of group 2 and group 3 patients with elevated PVR that might benefit from a PAH targeted therapeutic agent?
• How can we accelerate translation of investigator initiated research to advance therapy and diagnosis of PAH?
  – Can we create a forum to allow industry to examine potential therapeutic targets and biomarkers discovered by investigators to enhance knowledge translation?
• Is there a need for more advanced and sensitive endpoints to evaluate progression and regression of PAH?
  – What is the role of routine cardiac MRI, MRI lung perfusion, exercise right heart catheterization, and measurements of right ventricle-pulmonary artery coupling?
• Patients are not consulted routinely in the design of clinical trials of PAH therapies
  – What endpoints are most meaningful to PAH patients?
  – Can PAH patients advocate for the funding of investigator initiated therapies?
• PAH is a very heterogeneous syndrome
  – How can we begin to apply a personalized medicine approach to these patients?
  – Is the best level to personalize PAH therapy identified at the genomic, epigenomic, or proteomic level?

Epidemiology

• Much of the epidemiology in PAH comes from registries and specialized clinics
  – What is the epidemiology of PAH in population based studies in adults and children?
  – Should we routinely compare the epidemiology of group 1 disease with the other pulmonary hypertension groups?
• Does the changing epidemiology of group 1 pulmonary hypertension (with more older, obese patients) reflect misclassification of group 2 pulmonary hypertension patients with HFpEF?
• What is the basis for the female preponderance in PAH?
• How best can regional and national PAH registries be standardized and shared?

Basic and translational science

• How does the BMPR2 pathway intersect with other important PAH pathways?
  – Why is BMPR2 downregulated in patients and animals lacking gene mutations?
  – Does the BMPR2 pathway offer viable therapeutic targets?
• What classes of drugs should be the focus of phase I clinical trials in PAH?
  – Candidates include anti-inflammatory agents, immunomodulators, metabolic modulators that target Warburg metabolism, agents targeting fibrosis, agents modulating mitochondrial dynamics, inhibitors of rho kinase, and agents that enhance expression of or function of BMPR2
• Although animal models of PAH are imperfect, it may be that the experimental design of preclinical studies is more to blame for false positive reports of therapeutic benefits that may not be reproducible in patients.
  – What is the role for randomization and blinding in preclinical pulmonary hypertension studies?
  – Should papers assessing preclinical therapies for pulmonary hypertension be required to include measures of pulmonary arterial pressure, cardiac output, and/or PVR?
  – Is there a standard duration of observation and ethically acceptable mortality surrogate that should be used in preclinical studies to avoid falsely optimistic assessment of drug benefit?
  – Can we create standardized protocols and models for preclinical assessment of PAH to make findings more robust and representative of the human disease?
• How can we better share DNA, mRNA, proteins, cells, and tissues from patients with PAH to advance discovery?
  – Should resource sharing always result in authorship, or would a fee for service repository of biomaterials be beneficial?
• Are the peripheral vasculature, right ventricular coronary vasculature, or skeletal muscles directly affected in PAH?

Are circulating inflammatory, autoimmune, or epigenetic factors directly targeting tissues beyond the pulmonary vasculature in PAH?
Box 5 | Differences between PAH guideline documents

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warburg metabolism and altered mitochondrial dynamics</td>
<td>Inhibitors of pyruvate dehydrogenase kinase (eg, dichloroacetate); inhibitors of fatty acid oxidation (eg, trimetazidine and ranolazine); anti-fission/pre-fusion therapies</td>
</tr>
<tr>
<td>Nonsense mutation (premature termination codon)</td>
<td>Ataluren (PTC-124)</td>
</tr>
<tr>
<td>Missense mutation</td>
<td>Chemical chaperones (eg, 4-PBA)</td>
</tr>
<tr>
<td>Independent of BMPR2 mutation status</td>
<td>Recombinant BMP9; FK506 (tacrolimus); hydroxychloroquine</td>
</tr>
<tr>
<td>Epigenetic mechanisms of disease</td>
<td>DNMT inhibitors; HDAC inhibitors (eg, valproic acid); micro-RNA therapies; miR mimics, antagon-miRs</td>
</tr>
<tr>
<td>Immune dysfunction</td>
<td>Anti-interleukin 6 receptor antibodies, immunosuppressants, leukotriene-A4 hydrolase inhibitors</td>
</tr>
<tr>
<td>Right ventricular dysfunction</td>
<td>Anti-angiogenic right ventricle therapies, adrenergic modulations (GRK2 inhibitors, β-adrenergic blockers)</td>
</tr>
</tbody>
</table>

6-PBA—4-phenylbutyrate; BMPR II—type II bone morphogenetic protein receptor; BMPR2—bone morphogenetic protein receptor; DNMT—DNA methyltransferases; GRK=G protein-coupled receptor kinase; HDAC—histone deacetylases; PTC=premature termination codons.

less likely to improve their RVEF after starting PAH targeted therapy. Male and female patients showed a similar reduction in PVR one year after starting PAH targeted therapy, but RVEF improved in female patients, whereas it deteriorated in male patients. 12 This difference accounted for much of the male survival disadvantage. Even healthy people have differences in right ventricular function, such that men have lower RVEF than women (by about 4%), despite greater right ventricular mass (approximately 8%) and volumes. 257 Beyond the adaptive response of the right ventricle, other important sex differences in the pathogenesis of PAH exist in both the development and progression of vascular obstruction and the responsiveness to PAH targeted therapy. 238 These are beyond the scope of our review, and the interested reader is referred to the literature. 296-105 239

Guidelines

Three main guideline statements about the care of adult patients with pulmonary hypertension are available. 14 46 240 Differences between guidelines documents are reviewed in box 5.
Emerging therapies
Table 7 summarizes emerging therapies for PAH that address novel targets.

Conclusions
Tremendous advances have been made in the diagnosis and management of PAH. As a result, patients have access to four classes of PAH specific agents. However, none of these agents is curative and all are expensive. A concerning trend in clinical trials is to use composite endpoints that include mortality and imply survival benefits when outcomes are driven by reductions in hospital admission or improved functional capacity. Arguably, the available PAH specific therapies do not target key features of this syndrome, including its neoplastic, mitochondrial-metabolic, inflammatory, and fibrotic phenotypes. Although basic science studies have clarified several key molecular pathways that underlie PAH, the translation of these studies into clinically available biomarkers and new therapeutic agents has been slow, and no clear path exists to move these agents into clinically available biomarkers and new therapeutic agents. It is increasingly clear that these mechanisms intersect, offering new therapeutic targets.

Contributors
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Competing interests
We have read and understood the BMJ policy on declaration of interests and declare the following interests: TT received a modest honorarium from Gilead and Actelion for participating in a declaration of interests and declare the following interests: TT received a modest honorarium from Gilead and Actelion for participating in an advisory board.

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