Treatment of pulmonary arterial hypertension with targeted therapies

Dermot S. O'Callaghan, Laurent Savale, David Montani, Xavier Jaïs, Olivier Sitbon, Gérald Simonneau and Marc Humbert

Abstract | Pulmonary arterial hypertension (PAH) is a rare disorder characterized by progressive obliteration of the pulmonary microvasculature that results in elevated pulmonary vascular resistance and premature death. Although no cure exists for PAH, improved understanding of the pathobiological mechanisms of this disease has resulted in the development of effective therapies that target specific aberrant pathways. Agents that modulate abnormalities in the prostacyclin, endothelin, and nitric oxide pathways have been shown in randomized, controlled studies to confer improvements in functional status, pulmonary hemodynamics, and possibly even slow disease progression. Several additional pathways believed to play an important role in the pathogenesis of PAH have been identified as potentially useful therapeutic targets and a number of investigative approaches focusing on these targets are in active development. In this Review, we highlight the pharmacological agents currently available for the treatment of PAH and discuss potential novel strategies.

O'Callaghan, D. S. *et al. Nat. Rev. Cardiol.* **8**, 526–538 (2011); published online 19 July 2011; doi:10.1038/nrcardio.2011.104

Introduction

Pulmonary arterial hypertension (PAH) is a rare disorder, with a prevalence in the order of 15–50 patients per million of the population.[1,2] The condition can occur in an idiopathic form or develop in the context of other conditions (Box 1). PAH is characterized by endothelial cell dysfunction, endothelial and pulmonary artery smooth muscle cell (PASMC) proliferation, pulmonary vasoconstriction, and *in situ* thrombosis, which leads to sustained increases in pulmonary vascular resistance (PVR) and pulmonary arterial pressure, culminating in progressive right ventricular dysfunction and death.[3] The diagnosis of PAH is made by invasive hemodynamic assessment at right heart catheterization and confirmed by a resting mean pulmonary arterial pressure (mPAP) of ≥25 mmHg and a normal (≤15 mmHg) pulmonary capillary wedge pressure.[4]

Over the past quarter of a century, PAH has been transformed from a disease that was almost universally rapidly fatal, owing to the lack of treatment strategies, to one for which several therapeutic options are now available (Figure 1). In 1991, D'Alonzo *et al.* reported 1-year and 3-year survival rates of 68% and 48%, respectively, in a cohort of 194 patients with IPAH from 32 centers in the USA.[5] By contrast, results from a study published 20 years later by Humbert *et al.* revealed 1-year and 3-year survival rates of 83% and 58%, respectively, among 190 cases of incident and prevalent idiopathic, familial, or anorexigen-associated PAH in the French National Registry.[6] These findings suggest that, although survival has improved in the modern management era, PAH remains a progressive disease with unacceptably high mortality, particularly in incident cases of the disease. Randomized, clinical trials have demonstrated that the use of 'PAH-specific therapies' confers sustained improvements in important measures of clinical status, such as exercise capacity, functional class, and pulmonary hemodynamics, and reduces rates of clinical detererioration.[7,8] Data from the NIH registry is often used to contrast the actual survival of patient cohorts receiving modern PAH therapies with those that could have been predicted on the basis of older therapies, such as anticoagulants, oral vasodilators, diuretics, and lung transplantation. However, comparing outcomes of patients recruited to modern-day clinical trials of investigational PAH therapies with those of historical controls from the NIH registry has important limitations. The results of several studies published in 2010 suggest that the regression equation that was devised to predict a patient's likelihood of survival according to baseline hemodynamic measurements (the 'NIH equation') might not be accurate in determining prognosis for patients in the modern treatment era.[6,9,10] In 2010, novel equations were proposed in populations with incident idiopathic, heritable, and drug-induced PAH.[10,11] Such equations are probably more useful comparators when populations of patients with incident idiopathic PAH are exclusively analyzed.

Substantial changes in current clinical practice could also contribute to the improved outcomes of patients receiving investigational treatments for PAH in the

Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre F-94276; Service de Pneumologie, Hôpital Antoine Béclère, AP-HP, Clamart F-92140; INSERM U999, Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson F-92350, France (**D. S. O'Callaghan**, **L. Savale**, **D. Montani**, **X. Jaïs**, **O. Sitbon**, **G. Simonneau**, **M. Humbert**).

Correspondence to: M. Humbert marc.humbert@abc.aphp.fr

Competing interests
L. Savale declares associations with the following companies: Actelion, Novartis, and Pfizer. X. Jaïs declares associations with the following companies: Actelion, GlaxoSmithKline, Lilly, and Pfizer. D. Montani, O. Sitbon, G. Simonneau, and M. Humbert declare associations with the following companies: Actelion, Bayer–Schering, GlaxoSmithKline, Lilly, Novartis, Pfizer, and United Therapeutics. See the article online for full details of the relationships. D. S. O'Callaghan declares no competing interests.

setting of randomized studies. In particular, 'conventional' or 'background' therapies are now used in a more appropriate manner. For example, current best practice invokes a far more judicious application of traditional vasodilator therapy, by restricting the use of calcium-channel antagonists to patients with a confirmed acute vasodilator response to inhaled nitric oxide (NO) or other short-acting vasodilators at right heart catheterization. In addition, oral anticoagulants are now more-broadly used in the absence of compelling contraindications.[12]

Despite the tremendous strides that have been made in the field of PAH therapeutics, no cure for the disease exists. Further innovations are desperately needed as many patients inevitably progress despite treatment. In this Review, we discuss the various approaches used for the medical management of PAH and highlight potential novel targets under active investigation.

Key points

- Pulmonary arterial hypertension is a complex, rapidly progressive, and incurable disease
- The past decade has witnessed a remarkable increase in the number of available treatments for pulmonary arterial hypertension that can confer meaningful improvements in important clinical end points
- Currently licensed therapies for pulmonary arterial hypertension target abnormalities in the endothelin, prostacyclin, and nitric oxide signaling pathways
- Escalation of therapy using combination regimens is recommended for patients with pulmonary arterial hypertension who continue to exhibit evidence of disease progression
- Progress in basic and clinical research on pulmonary arterial hypertension has led to improved understanding of disease pathogenesis and identification of a host of novel therapeutic targets

Box 1 | Clinical classification of pulmonary arterial hypertension[3]

Idiopathic PAH

Heritable PAH:

- Mutations in bone morphogenetic protein receptor type II (*BMPR2*)
- Mutations in activin A receptor type II-like 1 (*ACVRL1*) or endoglin (*ENG*), with or without hereditary hemorrhagic telangiectasia
- Mutations in unknown gene

Drug-induced and toxin-induced PAH

PAH associated with:

- Connective tissue diseases
- HIV infection
- Portal hypertension
- Congenital heart diseases
- Schistosomiasis
- Chronic hemolytic anemia

Persistent pulmonary hypertension of the newborn

Currently available therapies

Prostanoids

Endothelium-derived prostaglandin I_2 (PGI_2), or prostacyclin, is a potent pulmonary vasodilator that also exerts antithrombotic, antiproliferative, antimitogenic, and immunomodulatory activity. In the pulmonary vasculature and serum of patients with PAH, prostacyclin synthase and prostacyclin metabolites are markedly reduced or absent.[13,14] The prostanoids are a family of stable prostacyclin analogs available in various formulations that have been developed for the treatment of PAH (Figure 2).

Epoprostenol

Epoprostenol is a synthetic sodium salt of naturally occurring PGI_2. In the early 1980s, this agent was the first prostanoid to be tested in patients with PAH. As the half-life of epoprostenol is <5 min, it requires the use of an infusion pump and an indwelling central venous catheter for continuous intravenous administration. Meticulous and frequent monitoring is required for patients being treated with epoprostenol because of the adverse-effect profile and the possibility of catheter-related infection. Commonly reported treatment-related effects are the result of systemic vasodilatation and include headache, flushing, jaw pain, and gastrointestinal upset. Interruption of epoprostenol delivery, either as a result of pump malfunction or catheter obstruction or damage, can result in potentially fatal rebound pulmonary hypertension.

The clinical efficacy of epoprostenol was examined in three unblinded studies.[15–17] In 1990, Rubin *et al.* reported that pulmonary hemodynamics improved in patients with idiopathic PAH (IPAH) after 2 months of epoprostenol therapy, whereas patients receiving conventional treatments (anticoagulants, oral vasodilators, and diuretics) experienced clinical deterioration.[15] A follow-on multicenter, uncontrolled trial of long-term epoprostenol use showed that improvements in exercise capacity and pulmonary hemodynamics were sustained for up to 1 year after initiation of treatment.[16] A larger subsequent study of epoprostenol among 81 patients with IPAH and NYHA functional class III–IV symptoms demonstrated a placebo-adjusted increase of 47 m in 6-min walk distance after 12 weeks.[17] In addition, a survival advantage with treatment was confirmed, as eight of the patients receiving conventional treatment (anticoagulants, oral vasodilators, diuretic agents, cardiac glycosides, and supplemental oxygen) died compared with none among the epoprostenol group.[17]

Subsequently published retrospective studies reported significantly improved survival in epoprostenol-treated patients compared with matched historical controls.[18,19] To date, epoprostenol remains the only PAH therapy to have been associated with a mortality benefit in a randomized clinical trial and, on this basis, was granted regulatory approval for treatment of patients with NYHA functional class III or IV symptoms. A novel formulation of epoprostenol (Veletri®, Actelion Pharmaceuticals Ltd, Allschwil, Switzerland) that is stable for up to 24 h without refrigeration was approved by the FDA in 2008.

Treprostinil

Treprostinil is a prostacyclin analog with greater stability and a longer (approximately 4 h) half-life than epoprostenol, which can be administered by the subcutaneous, intravenous, inhaled, or oral routes. The subcutaneous form of treprostinil is stable at room temperature and is delivered via a microinfusion pump. In a pivotal 12-week,

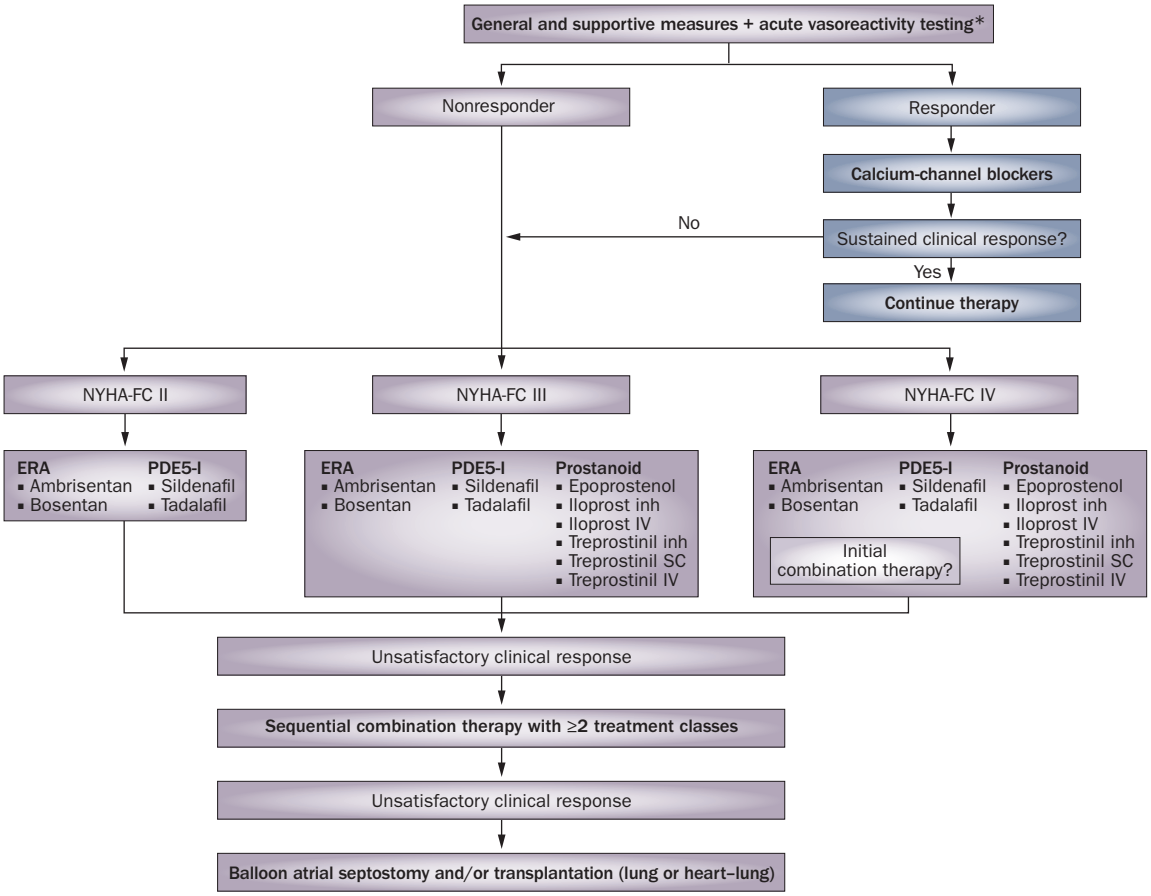

Figure 1 | Treatment algorithm for pulmonary arterial hypertension. *Licensed indications for individual agents with respect to functional class vary between countries. Abbreviations: ERA, endothelin receptor antagonists; FC, functional class; INH, inhaled; IV, intravenous; PDE5-I, phosphodiesterase type 5 inhibitors; SC, subcutaneous.

placebo-controlled randomized trial of 470 patients with PAH that was idiopathic or associated with connective tissue disease or congenital intracardiac shunt, treprostinil increased 6-min walk distance in a dose-dependent fashion and conferred improvements in symptoms, quality-of-life scores, and pulmonary hemodynamics.[20] Approval of treprostinil was granted in 2002 in the USA for patients with NYHA functional class II–IV symptoms. In Europe, approval was granted in 2005, but only for IPAH with associated NYHA functional class III symptoms. The major limitation of this formulation is infusion site pain, which occurs in the majority of patients. Furthermore, no firm evidence exists that treprostinil therapy improves survival in patients with PAH, irrespective of the method of administration.

Continuous intravenous infusion of treprostinil is only licensed for use in the USA and offers the advantage of less-frequent need for drug reservoir replacement (every 48 h compared with every 12–24 h for epoprostenol).[21] Transitioning patients from intravenous epoprostenol to intravenous treprostinil is feasible, although such an approach can be associated with mild hemodynamic deterioration.[22] Furthermore, concerns have been expressed regarding the relatively higher rate of Gram-negative blood-stream infections with intravenous treprostinil when compared with epoprostenol.[23] Switching patients from treprostinil to epoprostenol can also be necessary in cases of clinical worsening or intolerance of the former agent.[24]

Inhaled treprostinil has also been examined as a potential add-on therapy for PAH. In an initial open-label study, the addition of inhaled treprostinil was found to confer improvements in exercise capacity, modified NYHA functional class, and pulmonary hemodynamics among patients already receiving the endothelin receptor antagonist bosentan.[25] In the subsequent placebo-controlled TRIUMPH-1 study,[26] the effect of inhaled treprostinil in addition to either bosentan or sildenafil was examined among 235 patients with PAH, the majority of whom had persistent modified NYHA functional class III symptoms despite oral therapy (Table 1). After 12 weeks, treprostinil conferred a median improvement in postdose 6-min walk distance of 20 m and favorably impacted quality-of-life scores and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels. However, no improvements in time to clinical worsening, Borg dyspnea scores, or NYHA functional class were reported with the active drug. In 2009, the FDA granted approval for inhaled treprostinil for patients with PAH and NYHA functional class III symptoms; this agent is not yet licensed outside the USA.

The oral form of treprostinil has also been the subject of clinical studies. Investigators in the FREEDOM-C

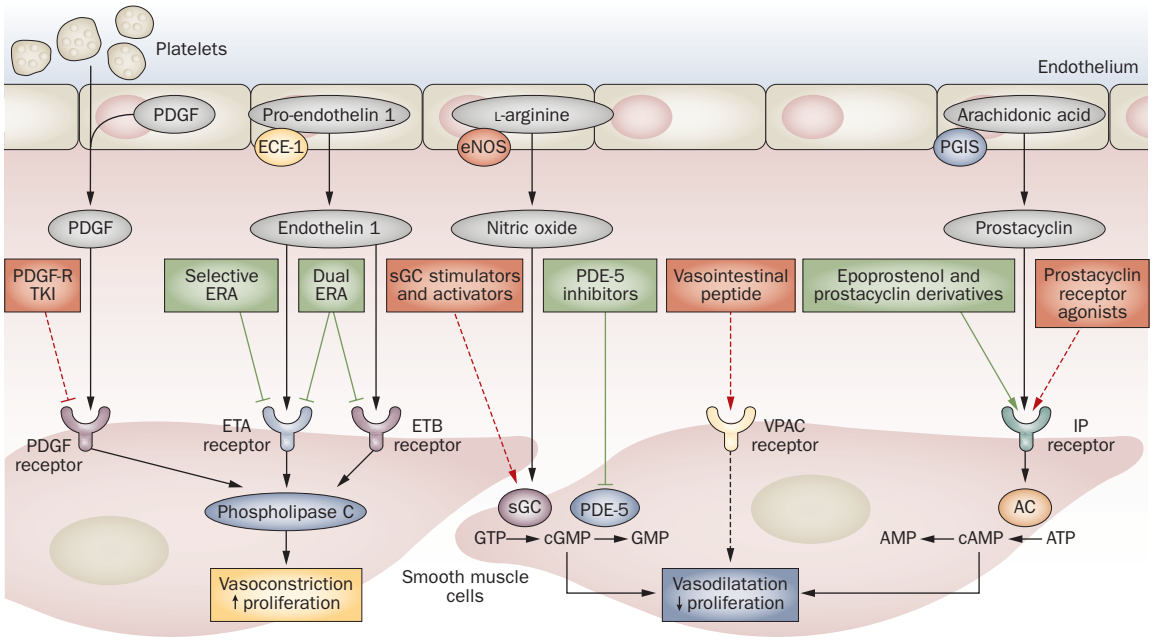

Figure 2 | Current and emerging targets and therapies for pulmonary arterial hypertension. Pulmonary artery smooth muscle cell therapeutic targets and corresponding licensed (green boxes) and investigational (red boxes) treatment approaches for pulmonary arterial hypertension. Arrows represent receptor stimulation, whereas terminated lines show receptor blockade. Abbreviations: AC, adenylate cyclase; cAMP, cyclic AMP; cGMP, cyclic GMP; ECE-1, endothelin converting enzyme 1; eNOS, endothelial nitric oxide synthase ; ETA, endothelin receptor type A; ETB, endothelin receptor type B; ERA, endothelin receptor antagonists; IP, prostaglandin I2; PDE-5, phosphodiesterase type 5; PDGF, platelet derived growth factor; PDGF-R TKI, PDGF receptor tyrosine kinase inhibitors; PGIS, prostaglandin I synthase; sGC, soluble guanylate cyclase; VPAC, vasointestinal peptide receptor.

trial[27] randomly assigned 354 patients on stable doses of other classes of oral PAH therapy (an endothelin receptor antagonist, a phosphodiesterase (PDE) type 5 (PDE-5) inhibitor, or both) to receive oral treprostinil, titrated to the maximum tolerated dose, or placebo. No statistically significant change in the primary end point of change in 6-min walk distance after 16 weeks was noted for treprostinil, although patients able to achieve higher treatment doses showed greatest improvement in exercise capacity.[27] Results are awaited from the FREEDOM-M study,[28] in which the safety and efficacy of oral treprostinil as monotherapy in patients with PAH is being evaluated.

Beraprost

Beraprost is another orally active prostanoid that has demonstrated modest efficacy in patients with PAH. In ALPHABET,[29] 130 patients with PAH were randomly assigned to receive oral beraprost (median dose 80 mg four times daily) or placebo. After 12 weeks, the difference between treatment groups in the mean change of 6-min walk distance was +25 m in favor of beraprost ($P = 0.036$). However, only patients with IPAH demonstrated significant improvements in exercise capacity. No statistically significant changes in hemodynamic indices or NYHA functional class were reported.[29] Findings from a subsequent study of beraprost, in which 116 patients with NYHA functional class II or III symptoms were evaluated, indicated that treatment increased exercise capacity and reduced risk of disease progression at 6 months, although efficacy was not sustained with extended duration of therapy.[30] Indeed, the effect of beraprost was no greater than placebo at 12 months. Currently, oral beraprost is licensed for use only in Japan and South Korea.

Iloprost

Iloprost is a stable prostacyclin analog developed for inhaled and intravenous administration. The pulmonary vasodilatory effects of inhaled iloprost last approximately 30–45 min. Regulatory approval for inhaled iloprost was granted in Europe in 2003 and by the FDA in late 2004 on the basis of the AIR study,[31] which was conducted at 37 European specialist centers. Treatment with inhaled iloprost was compared with placebo in 203 patients with PAH (IPAH or PAH associated with scleroderma or the use of anorexigens) or chronic thromboembolic pulmonary hypertension and associated NYHA functional class III or IV symptoms. Study participants received daily inhalations of 2.5 μg or 5.0 μg of iloprost (6–9 times per day) or placebo. At 12 weeks, 17% of iloprost-treated patients, compared with 5% of placebo-treated patients, reached the combined primary end point of improvement in functional class and 10% increase in 6-min walk distance in the absence of clinical deterioration or death. Postinhalation pulmonary hemodynamic values were significantly improved among those receiving iloprost ($P < 0.001$) and worse in the placebo cohort. The most-commonly observed adverse events were cough, flushing, jaw pain, and headache.[31] However, multiple daily nebulizations of iloprost, each of which can take up to 15 min to complete, are required for efficacy. Furthermore, in an open-label

Table 1 | Key phase II and III clinical trials of investigative PAH therapies since the 2009 ERS/ESC guidelines[3]

Study name	Phase	Agent	Therapeutic class	Administration route	Primary study end point	Study result
Prostacyclin pathway						
TRIUMPH-1[26]	III	Treprostinil	Prostanoid	Inhaled	6MWT	+20 m (P<0.001) at 12 weeks
FREEDOM-C[27]	III	Treprostinil	Prostanoid	Oral	6MWT	Negative study*
NIPPON[78]	II	Selexipag	Prostacyclin receptor agonist	Oral	Pulmonary hemodynamics	30% decreased PVR (P<0.01)
GRIPHON[79]	III	Selexipag	Prostacyclin receptor agonist	Oral	Time to clinical worsening	Study ongoing
Endothelin pathway						
SERAPHIN[65]	III	Macitentan	Tissue targeting ERA	Oral	Time to clinical worsening	Study ongoing
Nitric oxide pathway						
PATENT-1[90]	III	Riociguat	Soluble guanylate cyclase stimulator	Oral	6MWT	Study ongoing
Vasoactive intestinal peptide						
VIP[85]	II	Aviptadil	Vasointestinal peptide pathway	Inhaled	Pulmonary hemodynamics	Negative study‡
Tyrosine kinase inhibitor						
IMPRES[98]	III	Imatinib	Tyrosine kinase inhibitor	Oral	Time to clinical worsening	Study ongoing
Rho kinase pathway						
SiPHT[119]	I and II	Simvastatin	HMG Co-A reductase inhibitor	Oral	RV mass (by MRI)	Negative study§
ASA-STAT[120]	III	Simvastatin‖	HMG Co-A reductase inhibitor	Oral	6MWT	Negative study¶

*Placebo-corrected median change in 6MWT distance at week 16 of 11 m (P=0.072). ‡No reduction in PVR compared with placebo, either after a single inhalation or after 12 weeks of treatment. No changes in 6MWT, NT-pro BNP levels or functional class after 12 weeks. §Reduced RV mass at 6 months, but changes not sustained at 12 months. ‖A second arm of the study examining aspirin versus placebo was also negative using the same end point. ¶Trend toward worse 6MWT and dyspnea at 6 months, but study stopped early owing to futility in reaching primary end point. Abbreviations: 6MWT, 6-min walk test; ERA, endothelin receptor antagonist; ERS, European Respiratory Society; ESC, European Society of Cardiology; HMG Co-A, hydroxy-3-methyl-glutaryl coenzyme A; PVR, pulmonary vascular resistance; RV, right ventricle.

German study, relatively few patients maintained long-term clinical stability when inhaled iloprost was initiated as monotherapy.[32]

Nonrandomized study data suggest that intravenous iloprost has an efficacy profile similar to that of intravenous epoprostenol,[33,34] and has greater stability in solution and a longer plasma half-life of approximately 25 min.[35,36] Intravenous iloprost can be considered for patients with advanced PAH, although it has not been formally tested in a randomized, placebo-controlled trial. Moreover, a retrospective analysis of 79 patients with PAH who were treated with intravenous iloprost (introduced primarily because of clinical worsening despite initial inhaled iloprost) revealed a high rate of treatment failure and poor survival.[37] New Zealand remains the only country to have granted regulatory approval for intravenous iloprost.

Endothelin receptor antagonists

Endothelin 1 is a potent vasoconstrictor and PASMC mitogen that plays an important pathogenic role in PAH.[38] The various biologic actions of endothelin 1 are the result of activation of two distinct receptor isoforms, designated the endothelin A (ETA) and endothelin B (ETB) receptors. In pulmonary arteries, the ETA receptor is predominantly expressed on PASMCs, whereas the ETB receptor is predominantly localized on the vascular endothelium, with a relatively lesser expression on PASMCs. Binding of endothelin 1 to ETA and ETB receptors on PASMCs promotes vasoconstriction, whereas activation of ETB receptors on endothelial cells causes vasodilatation through increased prostacyclin and NO levels.[39,40]

Bosentan

Bosentan is a nonpeptide, pyrimidine derivative that binds to and irreversibly blocks both endothelin 1 receptor subtypes. Results from an initial placebo-controlled study involving 32 patients with IPAH or scleroderma-related PAH confirmed that bosentan conferred significant improvements in exercise capacity.[41] At the end of 16 weeks, bosentan-treated patients demonstrated an increase in 6-min walk distance of 76 m compared with placebo. Adverse events were reported with similar frequency between both groups. However, two patients receiving bosentan experienced a transient increase in the level of hepatic transaminases.

The efficacy of bosentan was confirmed in BREATHE-1,[42] in which 213 patients with NYHA functional class III and IV symptoms were randomly assigned to receive twice daily bosentan or placebo in a 2:1 ratio.

Two dosing schedules of bosentan were examined to assess the impact of dose ranging. After 16 weeks, placebo-adjusted changes in the primary efficacy end point of change in 6-min walk distance of +54 m and +33 m were observed in patients receiving 250 mg and 125 mg doses of bosentan, respectively, yielding an overall change of +44 m in favor of bosentan. No significant difference with respect to change in 6-min walk distance between the two tested bosentan doses was observed, confirming a lack of dose–response relationship. Improvements in NYHA functional class were reported in 42% of bosentan-treated patients compared with 30% in the placebo arm. In addition, bosentan-treated patients had overall greater improvements in dyspnea scores and delayed time to clinical worsening.[42] A trend toward higher frequency of liver injury with bosentan was confirmed in BREATHE-1,[42] with increases in levels of alanine aminotransferase, aspartate aminotransferase, or both to >3 times the upper limit of normal developing in 12% and 14% of patients in the 125 mg and 250 mg bosentan groups, respectively. Seven patients receiving bosentan experienced an increase in hepatic transaminase levels of >8 times the upper limit of normal, although these episodes were transient in most patients. However, three individuals randomly assigned to receive the 250 mg dose were withdrawn from the study before completion.[42]

Bosentan was granted regulatory approval by the FDA in 2001 and in Europe in 2002 on the basis of BREATHE-1.[42] Treatment is initiated at a dose of 62.5 mg twice daily for 4 weeks and then increased to the licensed maintenance dose of 125 mg twice daily.[3] Monthly monitoring of liver transaminases is mandatory in patients treated with endothelin receptor antagonists. Postmarketing surveillance of bosentan has showed that elevated aspartate aminotransferase or alanine aminotransferase levels >3 times the upper limit of normal are not uncommon during therapy (annual rate of 10.1%) and may necessitate dose reduction or treatment discontinuation.[43]

The design of the initial bosentan studies precluded any direct analysis of a potential impact on mortality. However, results from subsequently published studies suggest that long-term treatment confers durable improvements in exercise capacity, modified NYHA functional class, pulmonary hemodynamics, and possibly survival.[44–46] Data from EARLY[47] showed that treatment with bosentan in patients with mildly symptomatic PAH conferred improvements in PVR and significantly delayed time to clinical worsening compared with placebo. As a result, treatment with bosentan is now also licensed for patients with NYHA functional class II symptoms.

The impact of bosentan in populations with other forms of PAH has also been examined. Significant improvements in pulmonary hemodynamic parameters associated with treatment were reported in a small randomized, placebo-controlled trial of patients with Eisenmenger's syndrome,[48] and beneficial effects were also reported in children with PAH,[49] in patients with HIV-related PAH,[50,51] and in those with portopulmonary hypertension.[52,53] Data from randomized, prospective studies in these specific populations, however, are lacking.

Ambrisentan

Selective ETA receptor antagonism has been suggested to be preferable to nonselective ETA or ETB blockade as, theoretically, the former approach would block the release of pulmonary vasoconstrictors while preserving endothelin 1-mediated vasodilatory and antimitogenic activity through unimpeded activation of the endothelial ETB receptor.[54] This hypothesis led to the development and clinical evaluation of the selective ETA receptor antagonist class as a PAH treatment.[55]

Ambrisentan is an orally active, highly selective ETA receptor antagonist administered once daily. After demonstrating a favorable safety and efficacy profile in an initial dose-ranging study,[56] ambrisentan was the subject of two randomized, double-blind, placebo-controlled studies. ARIES 1 and 2[57] were essentially identical, other than the locations of participating study centers (North America for ARIES-1 and Western Europe for ARIES-2) and doses of ambrisentan tested (5 mg and 10 mg in ARIES-1; 2.5 mg and 5 mg in ARIES-2). In total, 394 patients with IPAH or PAH associated with anorexigen exposure, connective tissue disease, or HIV infection were evaluated. Combined analysis of the two studies demonstrated that ambrisentan treatment improved 6-min walk distance after 12 weeks compared with placebo (range +31 m to +59 m). The delay in time to clinical worsening was significantly longer among patients randomly assigned to receive ambrisentan in ARIES-2, but not in ARIES-1, whereas improvements in modified NYHA functional class were observed among those receiving the study drug in ARIES-1 only. A favorable impact on several secondary end points, including breathlessness scores, Short Form-36 Health Survey and BNP levels were also observed among patients receiving ambrisentan. The most commonly recorded adverse events were peripheral edema, headache, and nasal congestion. No significant increases in liver transaminase levels were observed in the ambrisentan arm.[57] The low rate of hepatic injury was confirmed by McGoon *et al.* in 2009, who followed up 36 patients receiving ambrisentan after discontinuing bosentan, sitaxentan, or both owing to increased transaminase levels.[58] No elevations in levels of hepatic transaminases requiring ambrisentan discontinuation were observed (median drug exposure of 102 weeks).[58]

Patients from these studies were eligible for inclusion in the long-term ARIES-E (extension) study.[59] After 1 year of treatment, 95% of patients who received ambrisentan were still alive, with 93% continuing ambrisentan monotherapy. After 2 years of treatment, mean increase in 6-min walk distance was maintained at +23 m and +28 m for the 5 mg and 10 mg doses, respectively.[59] Ambrisentan was granted regulatory approval by the FDA in 2007 and in Europe in 2008 for patients with PAH-related NYHA functional class II, III (Europe and North America), and IV (North America only) symptoms.

Sitaxentan

The selective ETA receptor antagonist sitaxentan was approved in Europe and Canada in 2006 and in

Australia in 2007 on the basis of favorable results from the STRIDE-1[60] and STRIDE-2[61] trials. Data from these studies suggested a lower rate of liver transaminase increases with sitaxentan compared with bosentan. However, a number of deaths attributed to acute liver failure were subsequently reported in patients receiving sitaxentan[62,63] and the drug was withdrawn from the market worldwide in December 2010.

Macitentan

Macitentan is a novel, highly potent, tissue-targeting endothelin receptor antagonist characterized by high lipophilicity that is under investigation.[64] By remaining active in local tissue environments, macitentan has the theoretical advantage of an extended functional *in vivo* half-life. In the phase III placebo-controlled SERAPHIN trial,[65] the efficacy and safety of macitentan will be tested in approximately 750 patients with PAH. The primary objective in this study is to establish whether treatment with macitentan prolongs time to the first morbidity or mortality event. Results are expected in 2012.

Phosphodiesterase type 5 inhibitors

NO is a potent PASMC relaxant that exerts vasodilatory activity through upregulation of its associated downstream signaling molecule, cyclic GMP (cGMP), metabolism of which is dependent on the activation of a number of PDEs. Within the pulmonary circulation, PDE-5 is the most abundantly expressed isoform. Accordingly, therapeutic strategies that limit cGMP metabolism, thereby maintaining the beneficial vasodilatory effects of NO, are recognized to be of interest in the treatment of vascular diseases. PAH is associated with abnormally low levels of NO within the pulmonary endothelium, owing in part to the reduced expression of NO synthase in the lung microvasculature.[66] In this regard, inhibitors of PDE-5 have emerged as an important therapeutic class.

Sildenafil

The first large, randomized, placebo-controlled trial evaluating a PDE-5 inhibitor was the SUPER-1 study,[67] in which the effects of various doses of sildenafil were tested in 278 patients with IPAH or PAH related to connective tissue disease or surgically corrected congenital systemic-to-pulmonary shunts. The majority of study participants had NYHA functional class II and III symptoms. After 12 weeks of treatment, the mean placebo-adjusted changes in 6-min walk distance for the 20 mg, 40 mg, and 80 mg doses of sildenafil were +45 m, +46 m, and +50 m, respectively. Patients in the various sildenafil groups were also more likely to improve by at least one NYHA functional class and demonstrate improvement in pulmonary hemodynamics than those receiving placebo. Adverse events more frequently observed with sildenafil than placebo were headache, flushing, and dyspepsia. Long-term extension data from 222 patients who completed 1 year of sildenafil monotherapy at the 80 mg dose revealed sustained improvements in exercise capacity, suggesting a durable treatment effect.[67] In addition, at 3 years of follow-up, functional status was either improved or stable in 60% of patients enrolled in the SUPER-1 study,[67] and 46% has improved or stable 6-min walk distance. A second PAH therapy was added in 18% of patients and 3-year survival was estimated at 79%.[68] In the USA and Canada, treatment with sildenafil is licensed for patients with NYHA functional class II–IV symptoms, whereas approval in Europe was granted only for those with NYHA class II–III symptoms.

Tadalafil

Another PDE-5 inhibitor, tadalafil, was examined in the PHIRST-1 trial,[69] in which the investigators randomly assigned 405 patients who were either treatment-naive or already receiving bosentan therapy to placebo or one of several doses of tadalafil (2.5 mg, 10 mg, 20 mg, or 40 mg) for 16 weeks. At study completion, patients receiving tadalafil showed an overall mean placebo-corrected increase in 6-min walk distance of 33 m. However, this increase was dose-dependent, with only the 40 mg dose achieving the prespecified value for statistical significance for improvement. Those receiving this dose also had both delayed time to clinical worsening and fewer episodes of clinical worsening compared with placebo.[69] Analysis of comparative hemodynamic data from the 93 patients who underwent repeat cardiac catheterization showed that tadalafil conferred significant reductions in mPAP and PVR. Favorable effects with tadalafil 40 mg were confirmed among patients receiving background bosentan therapy, although improvements were less marked compared with the treatment-naive cohort.[70] Treatment-related changes in modified NYHA functional class were similar across all groups. Overall, treatment was well tolerated, with most adverse events being of mild-to-moderate severity. Tadalafil, which has the advantage of once-daily dosing, was granted regulatory approval for use in patients with PAH in North America and Europe in 2009.

Combination treatment

The various specific PAH therapies that act on the prostacyclin, endothelin, and NO pathways have been shown to favorably impact a variety of clinically relevant end points. However, disease progression is frequently observed despite the use of such treatments and employment of multidrug regimens in order to simultaneously target different targets is an appealing concept.[71] Accordingly, combination approaches using two or more agents from different drug classes are now recommended for patients with NYHA functional class III–IV symptoms who fail to improve after initiation of specific therapy, and also for individuals with NYHA class II symptoms who show clinical worsening with monotherapy.[3] If the patient's clinical status fails to improve sufficiently despite the introduction of a second therapeutic class of drug, triple combinations regimens may be considered. Nevertheless, there remains a lack of prospective studies clearly demonstrating that combination therapy confers long-term clinical benefits over monotherapy.

A frequently employed approach is the sequential addition of treatments in order to meet therapeutic targets[72]

and this strategy is advocated in current PAH guidelines.[3] However, further studies are needed to establish whether such a goal-oriented strategy is preferable to upfront double or and even triple combination approaches.[73] In this regard, the AMBITION study[74] is of particular interest; in this trial, the outcomes of three groups randomly assigned to receive first-line treatment with ambrisentan, tadalafil, or both will be compared.

Emerging treatment approaches

Prostacyclin receptor agonists

A promising therapeutic approach under investigation is the use of a nonprostanoid agonist to directly activate the prostacyclin IP receptor. Selexipag is a first-in-class orally-active prodrug metabolized to the highly selective prostacyclin receptor agonist MRE-269, which has a half-life of over 6 h.[75] Selexipag does not exhibit high affinity for the prostaglandin E receptor 3 (EP_3) and exerts similar vasodilatory activity on both large and small pulmonary arterial branches.[76] These properties are likely to account for the greater vasodilatory activity observed with selexipag than with beraprost and iloprost.[76] Preclinical study results showed that twice-daily administration of selexipag attenuates right ventricular hypertrophy, improves pulmonary hemodynamics, and significantly increases survival in monocrotaline-treated rats.[76] In a microdosing study using 100 μg of selexipag in healthy, white, male volunteers, headache was the most commonly reported adverse event.[77] A 2010 report from a phase IIa study, involving 43 patients with PAH, showed that treatment with selexipag conferred significant improvements in PVR values compared with placebo.[78] A numerical improvement in 6-min walk distance was also observed. A phase III randomized trial (GRIPHON)[79] to examine the effect of selexipag on morbidity and mortality in PAH is underway.

Vasointestinal peptide

Vasointestinal peptide (VIP, or its analog aviptadil) is a 28-amino-acid hormone that exerts vasodilatory, antiproliferative, and anti-inflammatory effects. When VIP binds to its associated receptors, vasoactive intestinal polypeptide receptor 1 ($VPAC_1$) and $VPAC_2$, downstream activation of the cyclic AMP (cAMP) and cGMP second messenger systems occurs, leading to modulation of vascular tone.[80,81] In murine models, deletion of the *Vip* gene results in pulmonary vascular remodeling and severe pulmonary hypertension,[82] whereas *Vip*-knockout mice treated with VIP demonstrate attenuated vascular changes and reduced right ventricular remodeling.[82] Patients with PAH show reduced lung and serum VIP levels and upregulated pulmonary artery expression of both VIP receptor subtypes,[83] suggesting that targeting VIP might be a useful therapeutic approach.

An initial pilot study demonstrated that inhaled aviptadil improved exercise capacity, dyspnea, and pulmonary hemodynamics among eight treatment-naive patients with IPAH.[83] A subsequent study that evaluated 20 patients with pulmonary hypertension of various etiologies demonstrated that inhalation of 100 μg of aviptadil was associated with transient pulmonary vasodilatation and increased stroke volume and mixed venous oxygen saturation.[84] A modest increase in cardiac output was also observed. However, results from an as-yet unpublished double-blind, placebo-controlled, dose-finding phase II study involving 56 patients with PAH already treated with endothelin receptor antagonists, PDE-5 inhibitors, or both showed no significant effects on exercise capacity or pulmonary hemodynamics after addition of inhaled aviptadil.[85]

sGC stimulators and activators

Endothelium-derived NO regulates vascular homeostasis through PASMC relaxation via the activation of the second messenger cGMP. The clinical benefits associated with the PDE-5 inhibitor class has led to interest in testing whether other agents that modulate NO signaling might be similarly beneficial in PAH. Riociguat is a first-in-class drug that augments cGMP biosynthesis through direct stimulation of the enzyme soluble guanylate cyclase (sGC) promoting vasodilatation by direct stimulation of sGC in an NO-independent fashion and by sensitization of sGC to low endogenous NO levels.[86] Since the therapeutic effect of PDE-5 inhibitors is dependent on baseline NO expression (levels of which are typically reduced in PAH)[87] treatments that act directly on sGC could potentially have a greater efficacy than PDE-5 inhibitors.

An initial study found that oral riociguat attenuated acute hypoxic pulmonary vasoconstriction in mice in a dose-dependent fashion and improved established monocrotaline-induced PAH in rats.[88] Results from a multicenter, open-label, uncontrolled phase II trial involving 75 patients with PAH ($n = 33$) and chronic thromboembolic pulmonary hypertension ($n = 42$) showed that 12 weeks of oral riociguat conferred improvements in symptoms, NYHA functional class, exercise capacity, NT-proBNP level, and pulmonary hemodynamics.[89] Treatment was well-tolerated overall up to the highest planned 2.5 mg dose. In an ongoing randomized, placebo-controlled phase III study of riociguat (PATENT-1),[90] which has a primary outcome of change in exercise capacity, 462 patients with PAH will be enrolled. Preliminary results are expected in 2011.

Tyrosine kinase inhibitors

Platelet-derived growth factor (PDGF) is a potent endothelial and smooth muscle mitogen that contributes to abnormal pulmonary vascular remodelling.[91] Because the lungs of patients with PAH are characterized by increased expression of PDGF receptors compared with healthy controls,[92] treatments that modify PDGF signaling have been proposed to be potentially beneficial. Imatinib is an orally active PDGF receptor tyrosine kinase inhibitor currently licensed for the treatment of chronic myelogenous leukemia and c-Kit-positive gastrointestinal tumors that can also reverse experimental pulmonary hypertension.[93] Case reports have described favorable clinical and hemodynamic responses after imatinib was introduced as a therapeutic adjunct in patients with

advanced disease receiving other PAH-specific therapies,[94,95] or in patients with PAH who also had chronic myelogenous leukemia.[96]

In 2010, Ghofrani *et al.* reported the results from a pilot study involving 59 patients with PAH who had NYHA functional class II–IV symptoms despite treatment with prostanoids, endothelin receptor antagonists, PDE-5 inhibitors, or combinations thereof.[97] Imatinib treatment failed to improve 6-min walk distance compared with placebo, although patients in the treatment group showed statistically significant hemodynamic improvements.[97] Because post-hoc analyses in this study showed that patients with more-marked hemodynamic impairment appeared to respond best to imatinib, enrollment for the phase III trial has been limited to patients with PVR >800 dynes.s.cm^{-5}. Enrollment of >200 patients in IMPRES[98] has now been completed, and preliminary results are expected in 2011.

Sorafenib is a multikinase inhibitor with a wider spectrum of tyrosine kinase activity than imatinib that has been shown to attenuate pulmonary vascular remodelling and hemodynamic changes in rat models of pulmonary hypertension.[99] In a 16-week dose-finding, phase Ib study involving 12 patients with PAH who were receiving parenteral prostanoids, with or without associated sildenafil, oral sorafenib conferred increases in exercise capacity and echocardiographically-estimated right ventricular ejection fraction.[100] Moderate cutaneous reactions and alopecia were the most common adverse treatment effects. Of note, hemodynamic measurements indicated a reduction in cardiac output on therapy,[100] emphasizing possible cardiac effects of agents that block vascular endothelial growth factor. Indeed, with the emergence of tyrosine kinase inhibitors as a potential therapy in PAH, the benefit:risk ratio of this class of agent will need careful assessment. In particular, concerns have been raised about potential cardiac toxicity, especially in patients with pre-existing heart disease.[101,102]

Nilotinib is another orally active tyrosine kinase inhibitor used as treatment for chronic myelogenous leukemia that is currently being assessed in a multicenter, phase II trial as a potential therapy for PAH.[103] The investigators plan to compare three doses of nilotinib with placebo among 66 patients with NYHA class II–III symptoms, with change in PVR established as the primary end point.

Hitherto, the potential beneficial effects of tyrosine kinase inhibitors in patients with PAH have been attributed primarily to inhibition of vascular smooth muscle cell proliferation and pulmonary arterial remodeling. However, data exist to suggest that, in animal models at least, this class of agent might additionally exert potent pulmonary vasodilatory activity though attenuation of pulmonary artery smooth muscle contraction.[104] In this regard, Abe *et al.* have demonstrated that intravenous administration of imatinib reduces right ventricular systolic pressure in hypoxia-associated pulmonary hypertensive rodents in a fairly pulmonary-selective fashion.[104] The investigators speculated that this effect might occur through inhibition of a kinase or signaling pathway that regulates Ca^{2+} sensitivity, such as Rho kinase (ROCK).

Other potential targets

A number of other targets and pathways for the treatment of PAH are under active investigation. Serotonin contributes to the pathophysiology of pulmonary hypertension by promoting proliferation of PASMCs and adventitial fibroblasts.[105] Elevated plasma serotonin levels have been reported in patients with IPAH,[106] and individuals exposed to fenfluramine derivatives—anorexigens that increase plasma serotonin levels—are at increased risk of the disease.[107,108] Blockade of the serotonin transporter might thus be a useful strategy in the treatment of PAH. In addition, the serotonin receptor 2B (5-HT_{2B}) is another possible target in PAH and a number of 5-HT_{2B} antagonists are in clinical development. In this regard, beneficial effects with terguride[109] and PRX-08066[110] have been shown in monocrotaline-induced PAH in rat models.

Activation of RhoA/ROCK signaling leads to vasoconstriction, vascular remodeling, and endothelial dysfunction. Inhibition of this pathway using the ROCK inhibitor fasudil in animal models of PAH has yielded promising results.[111] Studies in patients with PAH show favorable acute hemodynamic effects with the inhaled[112] and intravenous[113] formulations of fasudil, and evidence exists of increased ROCK activity in platelets, pulmonary arteries, and lungs in this population.[114,115] Rho signaling activation is also inhibited by hydroxy-3-methyl-glutaryl coenzyme A inhibitors[116] (statins) and, in animal models, these agents have been shown to attenuate pulmonary hypertension.[117,118] However, in a small randomized study of 42 patients with PAH, the addition of simvastatin to oral PAH therapy did not confer sustained beneficial effects.[119] Furthermore, the NIH-sponsored ASA-STAT[120] was stopped early following enrollment of 65 patients because of futility for simvastatin in reaching the prespecified primary end point of change in 6-min walk distance at 6 months.

The use of bone-marrow derived endothelial progenitor cells (EPCs), a population of circulating proangiogenic cells that repair and regenerate blood vessels, may represent an innovative approach to improving endothelial dysfunction through restoration of damaged pulmonary microvasculature. Administration of EPCs transduced with human endothelial NO synthase, the enzyme responsible for NO synthesis, resulted in improvement in established monocrotaline-induced PAH in rats.[121] Favorable effects on exercise capacity and pulmonary hemodynamics following autologous EPC infusion in patients with PAH have been reported[122] and a further study in underway in Canada.[123]

General measures and supportive therapy

A number of important general measures are advocated for patients with PAH in order to provide relief from symptoms and prevent clinical worsening.[3] Recommendations for these interventions are, however, mostly based on expert consensus or on data from uncontrolled observational studies rather than from randomized, controlled trials. Limiting physical activity to avoid potentially dangerous abrupt increases in cardiac demand is advisable. Cardiopulmonary rehabilitation has

been studied and found to be safe in patients with PAH, although additional confirmatory data are needed before this strategy can be broadly recommended.[124]

Administration of medications that might potentially aggravate PAH (such as β-adrenergic receptor blockers[125] or sympathomimetics) or interfere with the metabolism of vitamin K-antagonist anticoagulation therapy should be avoided. Supplemental oxygen should be considered for individuals with resting or exercise-induced hypoxemia in order to maintain oxygen saturation levels at >90%. In addition, oxygen administration during air travel might be warranted, particularly for patients with advanced disease.[126] Vaccinations to prevent pneumococcal pneumonia and influenza are also advisable.

One of the classic pathological hallmarks of PAH is widespread *in situ* intrapulmonary microthrombosis. In addition, dysregulation of normal coagulation and fibrinolytic pathways is characteristic in these patients.[127] Therefore, in the absence of compelling contraindications, oral anticoagulant therapy is recommended in IPAH, familial PAH, and anorexigen-associated PAH as this approach has been shown to be beneficial in open-label studies.[128,129] An assessment of the potential risks and benefits of long-term anticoagulation should be made for PAH associated with other diseases, particularly when an increased risk of bleeding exists. The effect of diuretics on mortality in patients with PAH has not been systemically examined. Nevertheless, such treatments offer symptomatic benefit in those with right ventricular volume overload that is not controlled by dietary measures alone.

The additional hemodynamic stresses of pregnancy and labor are poorly tolerated in patients with PAH, being associated with an increased rate of potentially fatal clinical worsening.[130] Female patients of childbearing potential should, therefore, be counseled on appropriate contraceptive measures. Because effectiveness of hormonal contraception can be reduced by certain PAH-specific treatments, a combination of pharmacological and mechanical (for example, an intrauterine device) contraception is advised.

A minority of patients with IPAH, familial PAH, or anorexigen-related PAH demonstrate significant hemodynamic improvements after administration of acute vasodilators at right heart catheterization.[131] Approximately half of these 'responders' will benefit from treatment with high-dose calcium-channel blockers.[131] However, confirmation of a sustained clinical and hemodynamic improvement by repeat catheterization after initiation of these agents is mandatory. The choice of agent is determined by the patient's heart rate at baseline. Treatment with nifedepine or amlodipine is preferred for patients with relative bradycardia, whereas diltiazem is favored for those with relative tachycardia.

Surgery has a key role in the treatment of patients who have progressive disease despite medical management. Lung transplantation is indicated for end-stage disease, with bilateral sequential lung transplantation and heart–lung transplantation being the most common procedures performed in patients with PAH.[132] Balloon atrial septostomy can be considered for patients with intractable right heart failure, particularly in countries where access to PAH-specific drugs is restricted.[133]

Conclusions

With an ever-increasing number of targeted therapies becoming available for the treatment of PAH, the management of this disorder has become increasingly complex. Although published guidelines provide broad recommendations for optimum approaches according to type and severity of disease, many uncertainties remain. Clinicians must select the most appropriate agent or agents for individual patients; such choices are influenced by patient preferences and local economic and regulatory considerations. Routine monitoring of response to treatment by clinical, functional, and hemodynamic assessment is critically important in guiding therapeutic decision-making. When treatment goals are considered to be unmet, escalation of therapy is recommended. However, the optimal timing and type of combination strategies require further study. Several novel agents are currently being evaluated in clinical trials and might eventually be shown to confer additional clinical improvements for patients with PAH.

Review criteria

Data and other information used in this review were derived from articles selected after a search of the NCBI/PubMed database for pertinent papers on therapy for pulmonary arterial hypertension. Key search terms included "endothelin", nitric oxide", "prostacyclin", "pulmonary arterial hypertension", and "treatment". The literature search was restricted to papers written in the English language published between 1980 and 2010. In addition, reference lists from identified publications were used as a cross-reference, particularly in relation to novel therapeutic approaches.

1. Humbert, M. *et al.* Pulmonary arterial hypertension in France: results from a national registry. *Am. J. Respir. Crit. Care Med.* **173**, 1023–1030 (2006).
2. Peacock, A. J., Murphy, N. F., McMurray, J. J., Caballero, L. & Stewart, S. An epidemiological study of pulmonary arterial hypertension. *Eur. Respir. J.* **30**, 104–109 (2007).
3. Galiè, N. *et al.* Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur. Respir. J.* **34**, 1219–1263 (2009).
4. Badesch, D. B. *et al.* Diagnosis and assessment of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **54**, S55–S66 (2009).
5. D'Alonzo, G. E. *et al.* Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann. Intern. Med.* **115**, 343–349 (1991).
6. Humbert, M. *et al.* Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* **122**, 156–163 (2010).
7. Humbert, M., Sitbon, O. & Simonneau, G. Treatment of pulmonary arterial hypertension. *N. Engl. J. Med.* **351**, 1425–1436 (2004).
8. Galiè, N. *et al.* A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. *Eur. Heart J.* **30**, 394–403 (2009).
9. Benza, R. L. *et al.* Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation* **122**, 164–172 (2010).
10. Thenappan, T. *et al.* Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. *Eur. Respir. J.* **35**, 1079–1087 (2010).
11. Humbert, M. *et al.* Survival in incident and prevalent cohorts of patients with pulmonary

arterial hypertension. *Eur. Respir. J.* **36**, 549–555 (2010).
12. Mehta, S. & Shoemaker, G. J. Improving survival in idiopathic pulmonary arterial hypertension: revisiting the "kingdom of the near-dead". *Thorax* **60**, 981–983 (2005).
13. Christman, B. W. *et al.* An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N. Engl. J. Med.* **327**, 70–75 (1992).
14. Tuder, R. M. *et al.* Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **159**, 1925–1932 (1999).
15. Rubin, L. J. *et al.* Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin (epoprostenol). Results of a randomized trial. *Ann. Intern. Med.* **112**, 485–491 (1990).
16. Barst, R. J. *et al.* Survival in primary pulmonary hypertension with long-term continuous intravenous prostacyclin. *Ann. Intern. Med.* **121**, 409–415 (1994).
17. Barst, R. J. *et al.* A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N. Engl. J. Med.* **334**, 296–302 (1996).
18. Shapiro, S. M. *et al.* Primary pulmonary hypertension: improved long-term effects and survival with continuous intravenous epoprostenol infusion. *J. Am. Coll. Cardiol.* **30**, 343–349 (1997).
19. McLaughlin, V. V., Shillington, A. & Rich, S. Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. *Circulation* **106**, 1477–1482 (2002).
20. Simonneau, G. *et al.* Continuous subcutaneous infusion of treprostinil, a prostacyclin analog, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* **165**, 800–804 (2002).
21. Laliberte, K., Arneson, C., Jeffs, R., Hunt, T. & Wade, M. Pharmacokinetics and steady-state bioequivalence of treprostinil sodium (Remodulin) administered by the intravenous and subcutaneous route to normal volunteers. *J. Cardiovasc. Pharmacol.* **44**, 209–214 (2004).
22. Gomberg-Maitland, M. *et al.* Transition from intravenous epoprostenol to intravenous treprostinil in pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **172**, 1586–1589 (2005).
23. Kallen, A. J. *et al.* Bloodstream infections in patients given treatment with intravenous prostanoids. *Infect. Control Hosp. Epidemiol.* **29**, 342–349 (2008).
24. Reisbig, K. A., Coffman, P. A., Floreani, A. A., Bultsma, C. J. & Olsen, K. M. Staggered transition to epoprostenol from treprostinil in pulmonary arterial hypertension. *Ann. Pharmacother.* **39**, 739–743 (2005).
25. Channick, R. N. *et al.* Safety and efficacy of inhaled treprostinil as add-on therapy to bosentan in pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **48**, 1433–1437 (2006).
26. McLaughlin, V. V. *et al.* Addition of inhaled treprostinil to oral therapy for pulmonary arterial hypertension: a randomized controlled clinical trial. *J. Am. Coll. Cardiol.* **55**, 1915–22 (2010).
27. Tapson, V. *et al.* Results of the FREEDOM-C Study: A Pivotal Study of Oral Treprostinil Used Adjunctively with an ERA and/or PDE5-Inhibitor for the Treatment of PAH [abstract A1040]. *Am. J. Respir. Crit. Care Med.* **179**, A17 (2009).
28. US NIH. ClinicalTrials.gov. FREEDOM -M: Oral treprostinil as monotherapy for the treatment of pulmonary arterial hypertension (PAH) [online], http://clinicaltrials.gov/ct2/show/NCT00325403 (2011).
29. Galiè, N. *et al.* Effects of beraprost sodium, an oral prostacyclin analog, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Cardiol.* **39**, 1496–1502 (2002).
30. Barst, R. J. *et al.* Beraprost therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **41**, 2119–2125 (2003).
31. Olschewski, H. *et al.* Inhaled iloprost for severe pulmonary hypertension. *N. Engl. J. Med.* **347**, 322–329 (2002).
32. Opitz, C. F. *et al.* Clinical efficacy and survival with first-line inhaled iloprost therapy in patients with idiopathic pulmonary arterial hypertension. *Eur. Heart J.* **26**, 1895–1902 (2005).
33. Higenbottam, T. W. *et al.* Treatment of pulmonary hypertension with the continuous infusion of a prostacyclin analog, iloprost. *Heart* **79**, 175–179 (1998).
34. Higenbottam, T., Butt, A. Y., McMahon, A., Westerbeck, R. & Sharples, L. Long-term intravenous prostaglandin (epoprostenol or iloprost) for treatment of severe pulmonary hypertension. *Heart* **80**, 151–155 (1998).
35. Olschewski, H. *et al.* Pharmacodynamics and pharmacokinetics of inhaled iloprost, aerosolized by three different devices, in severe pulmonary hypertension. *Chest* **124**, 1294–1304 (2003).
36. Opitz, C. F. *et al.* Assessment of the vasodilator response in primary pulmonary hypertension. Comparing prostacyclin and iloprost administered by either infusion or inhalation. *Eur. Heart J.* **24**, 356–365 (2003).
37. Hoeper, M. M. *et al.* Long-term outcome with intravenous iloprost in pulmonary arterial hypertension. *Eur. Respir. J.* **34**, 132–137 (2009).
38. Giaid, A. *et al.* Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.* **328**, 1732–1739 (1993).
39. Seo, B., Oemar, B., Siebenmann, R., von Segesser, L. & Lüscher, T. Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation* **89**, 1203–1208 (1994).
40. Hirata, Y. *et al.* Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. *J. Clin. Invest.* **91**, 1367–1373 (1993).
41. Channick, R. N. *et al.* Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet* **358**, 1119–1123 (2001).
42. Rubin, L. J. *et al.* Bosentan therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* **346**, 896–903 (2002).
43. Humbert, M. *et al.* Results of European post-marketing surveillance of bosentan in pulmonary hypertension. *Eur. Respir. J.* **30**, 338–344 (2007).
44. Sitbon, O. *et al.* Effects of the dual endothelin receptor antagonist bosentan in patients with pulmonary arterial hypertension: a 1-year follow-up study. *Chest* **124**, 247–254 (2003).
45. Sitbon, O. *et al.* Survival in patients with class III idiopathic pulmonary arterial hypertension treated with first line oral bosentan compared with an historical cohort of patients started on intravenous epoprostenol. *Thorax* **60**, 1025–1030 (2005).
46. McLaughlin, V. V. *et al.* Survival with first-line bosentan in patients with primary pulmonary hypertension. *Eur. Respir. J.* **25**, 244–249 (2005).
47. Galiè, N. *et al.* Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *Lancet* **371**, 2093–2100 (2008).
48. Galiè, N. *et al.* Bosentan therapy in patients with Eisenmenger syndrome: a multicenter, double-blind, randomized, placebo-controlled study. *Circulation* **114**, 48–54 (2006).
49. Rosenzweig, E. *et al.* Effects of long-term bosentan in children with pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **46**, 697–704 (2005).
50. Sitbon, O. *et al.* Bosentan for the treatment of human immunodeficiency virus-associated pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **170**, 1212–1217 (2004).
51. Degano, B. *et al.* Long-term effects of bosentan in patients with HIV-associated pulmonary arterial hypertension. *Eur. Respir. J.* **33**, 92–98 (2009).
52. Hoeper, M. *et al.* Bosentan therapy for portopulmonary hypertension. *Eur. Respir. J.* **25**, 502–508 (2005).
53. Hoeper, M. M. *et al.* Experience with inhaled iloprost and bosentan in portopulmonary hypertension. *Eur. Respir. J.* **30**, 1096–1102 (2007).
54. Opitz, C. F., Ewert, R., Kirch, W. & Pittrow, D. Inhibition of endothelin receptors in the treatment of pulmonary arterial hypertension: does selectivity matter? *Eur. Heart J.* **29**, 1936–1948 (2008).
55. Wu, C. *et al.* Discovery of TBC11251, a potent, long acting, orally active endothelin receptor-A selective antagonist. *J. Med. Chem.* **40**, 1690–1697 (1997).
56. Galiè, N. *et al.* Ambrisentan therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **46**, 529–535 (2005).
57. Galiè, N. *et al.* Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. *Circulation* **117**, 3010–3019 (2008).
58. McGoon, M. D. *et al.* Ambrisentan therapy in patients with pulmonary arterial hypertension who discontinued bosentan or sitaxsentan due to liver function test abnormalities. *Chest* **135**, 122–129 (2009).
59. Oudiz, R. J. *et al.* Long-term ambrisentan therapy for the treatment of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **54**, 1971–1981 (2009).
60. Barst, R. J. *et al.* Sitaxsentan therapy for pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **169**, 441–447 (2004).
61. Barst, R. J. *et al.* Treatment of pulmonary arterial hypertension with the selective endothelin-A receptor antagonist sitaxsentan. *J. Am. Coll. Cardiol.* **47**, 2049–2056 (2006).
62. Lavelle, A. *et al.* Sitaxentan-induced hepatic failure in two patients with pulmonary arterial hypertension. *Eur. Respir. J.* **34**, 770–771 (2009).
63. Lee, W. T. *et al.* Sitaxentan-related acute liver failure in a patient with pulmonary arterial hypertension. *Eur. Respir. J.* **37**, 472–474 (2011).
64. Iglarz, M. *et al.* Pharmacology of macitentan, an orally active tissue-targeting dual endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.* **327**, 736–745 (2008).
65. US NIH. ClinicalTrials.gov. Study of ACT-064992 on morbidity and mortality in patients with symptomatic pulmonary arterial hypertension [online], http://clinicaltrials.gov/ct2/show/NCT00660179?term=SERAPHIN&rank=2 (2010).
66. Giaid, A. & Saleh, D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.* **333**, 214–221 (1995).
67. Galiè, N. *et al.* Sildenafil citrate therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* **353**, 2148–2157 (2005).

68. Rubin, L. J. *et al.* Long-term treatment with sildenafil citrate in pulmonary arterial hypertension: SUPER-2. *Chest* doi:10.1378/chest.10-0969.
69. Galiè, N. *et al.* Tadalafil therapy for pulmonary arterial hypertension. *Circulation* **119**, 2894–2903 (2009).
70. Barst, R. J. *et al.* Tadalafil monotherapy and as add-on to background bosentan in patients with pulmonary arterial hypertension. *J. Heart Lung Transplant.* **30**, 632–643 (2011).
71. O'Callaghan, D. S. & Gaine, S. P. Combination therapy and new types of agents for pulmonary arterial hypertension. *Clin. Chest Med.* **28**, 169–185 (2007).
72. Hoeper, M. M., Markevych, I., Spiekerkoetter, E., Welte, T. & Niedermeyer, J. Goal-oriented treatment and combination therapy for pulmonary arterial hypertension. *Eur. Respir. J.* **26**, 858–863 (2005).
73. Galiè, N., Palazzini, M. & Manes, A. Pulmonary arterial hypertension: from the kingdom of the near-dead to multiple clinical trial meta-analyses. *Eur. Heart J.* **31**, 2080–2086 (2010).
74. US NIH. ClinicalTrials.gov. A study of first-line ambrisentan and tadalafil combination therapy in subjects with pulmonary arterial hypertension (AMBITION) [online], http://clinicaltrials.gov/ct2/show/NCT01178073 (2011).
75. Asaki, T., Hamamoto, T., Sugiyama, Y., Kuwano, K. & Kuwabara, K. Structure-activity studies on diphenylpyrazine derivatives: a novel class of prostacyclin receptor agonists. *Bioorganic Med. Chem.* **15**, 6692–6704 (2007).
76. Kuwano, K., Hashino, A., Noda, K., Kosugi, K. & Kuwabara, K. A long-acting and highly selective prostacyclin receptor agonist prodrug, 2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino] butoxy}-N-(methylsulfonyl)acetamide (NS-304), ameliorates rat pulmonary hypertension with unique relaxant responses of its active form, {4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino] butoxy}acetic acid (MRE-269), on rat pulmonary artery. *J. Pharmacol. Exp. Ther.* **326**, 691–699 (2008).
77. Kuwano, K. *et al.* 2-[4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-N-(methylsulfonyl) acetamide (NS-304), an orally available and long-acting prostacyclin receptor agonist prodrug. *J. Pharmacol. Exp. Ther.* **322**, 1181–1188 (2007).
78. Simonneau, G. *et al.* Efficacy, safety and tolerability of ACT-293987, a novel oral, non-prostanoid, prostaglandin I2 (IP) receptor agonist: results from a phase IIa study in pulmonary arterial hypertension (PAH) [abstract A2515]. *Am. J. Respir. Crit. Care Med.* **181**, B16 (2010).
79. US NIH. ClinicalTrials.gov. ACT-293987 in pulmonary arterial hypertension [online], http://clinicaltrials.gov/ct2/show/NCT01106014?term=GRIPHON&rank=1 (2011).
80. Murthy, K. S., Zhang, K. M., Jin, J. G., Grider, J. R. & Makhlouf, G. M. VIP-mediated G protein-coupled Ca^{2+} influx activates a constitutive NOS in dispersed gastric muscle cells. *Am. J. Physiol.* **265**, G660–G671 (1993).
81. Said, S. I. The vasoactive intestinal peptide gene is a key modulator of pulmonary vascular remodeling and inflammation. *Ann. N. Y. Acad. Sci.* **1144**, 148–153 (2008).
82. Said, S. I. *et al.* Moderate pulmonary arterial hypertension in male mice lacking the vasoactive intestinal peptide gene. *Circulation* **115**, 1260–1268 (2007).
83. Petkov, V. *et al.* Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J. Clin. Invest.* **111**, 1339–1346 (2003).
84. Leuchte, H. H. *et al.* Inhalation of vasoactive intestinal peptide in pulmonary hypertension. *Eur. Respir. J.* **32**, 1289–1294 (2008).
85. Galiè, N. *et al.* Effects of inhaled aviptadil (vasoactive intestinal peptide) in patients with pulmonary arterial hypertension (PAH) [abstract A2516]. *Am. J. Respir. Crit. Care Med.* **181**, B16 (2010).
86. Ghofrani, H. A. & Grimminger, F. Soluble guanylate cyclase stimulation: an emerging option in pulmonary hypertension therapy. *Eur. Respir. Rev.* **18**, 35–41 (2009).
87. Evgenov, O. V. *et al.* NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat. Rev. Drug Discov.* **5**, 755–768 (2006).
88. Schermuly, R. T. *et al.* Expression and function of soluble guanylate cyclase in pulmonary arterial hypertension. *Eur. Respir. J.* **32**, 881–891 (2008).
89. Ghofrani, H. A. *et al.* Riociguat for chronic thromboembolic pulmonary hypertension and pulmonary arterial hypertension: a phase II study. *Eur. Respir. J.* **36**, 792–799 (2010).
90. US NIH. ClinicalTrials.gov. A study to evaluate efficacy and safety of oral BAY63–2521 in patients with pulmonary arterial hypertension (PAH) [online], http://clinicaltrials.gov/ct2/show/NCT00810693?term=BAY63–2521&rank=1 (2011).
91. Barst, R. J. PDGF signaling in pulmonary arterial hypertension. *J. Clin. Invest.* **115**, 2691–2694 (2005).
92. Perros, F. *et al.* Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **178**, 81–88 (2008).
93. Schermuly, R. T. *et al.* Reversal of experimental pulmonary hypertension by PDGF inhibition. *J. Clin. Invest.* **115**, 2811–2821 (2005).
94. Ghofrani, H. A., Seeger, W. & Grimminger, F. Imatinib for the treatment of pulmonary arterial hypertension. *N. Engl. J. Med.* **353**, 1412–1413 (2005).
95. Patterson, K. C., Weissmann, A., Ahmadi, T. & Farber, H. W. Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension. *Ann. Intern. Med.* **145**, 152–153 (2006).
96. Souza, R., Sitbon, O., Parent, F., Simonneau, G. & Humbert, M. Long-term imatinib treatment in pulmonary arterial hypertension. *Thorax* **61**, 736 (2006).
97. Ghofrani, H. A. *et al.* Imatinib in pulmonary arterial hypertension patients with inadequate response to established therapy. *Am. J. Respir. Crit. Care Med.* **182**, 1171–1177 (2010).
98. US NIH. ClinicalTrials.gov. Imatinib in pulmonary arterial hypertension (IMPRES) [online], http://clinicaltrials.gov/ct2/show/NCT00902174 (2010).
99. Moreno-Vinasco, L. *et al.* Genomic assessment of a multikinase inhibitor, sorafenib, in a rodent model of pulmonary hypertension. *Physiol. Genomics* **33**, 278–291 (2008).
100. Gomberg-Maitland, M. *et al.* A dosing/cross-development study of the multikinase inhibitor sorafenib in patients with pulmonary arterial hypertension. *Clin. Pharmacol. Ther.* **87**, 303–310 (2009).
101. Kerkela, R. *et al.* Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat. Med.* **12**, 908–916 (2006).
102. Chen, M. H., Kerkela, R. & Force, T. Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics. *Circulation* **118**, 84–95 (2008).
103. US NIH. ClinicalTrials.gov. Efficacy, safety, tolerability and pharmacokinetics (PK) of nilotinib (AMN107) in pulmonary arterial hypertension [online], http://clinicaltrials.gov/ct2/show/NCT01179737?term=Nilotinib+pulmonary&rank=1 (2010).
104. Abe, K. *et al.* Tyrosine kinase inhibitors are potent acute pulmonary vasodilators in rats. *Am. J. Respir. Cell Mol. Biol.* doi:10.1165/rcmb.2010–0371OC.
105. Dempsie, Y. & MacLean, M. R. Pulmonary hypertension: therapeutic targets within the serotonin system. *Br. J. Pharmacol.* **155**, 455–462 (2008).
106. Hervé, P. *et al.* Increased plasma serotonin in primary pulmonary hypertension. *Am. J. Med.* **99**, 249–254 (1995).
107. Abenhaim, L. *et al.* Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N. Engl. J. Med.* **335**, 609–616 (1996).
108. Souza, R. *et al.* Pulmonary arterial hypertension associated with fenfluramine exposure: report of 109 cases. *Eur. Respir. J.* **31**, 343–348 (2008).
109. Dumitrascu, R. *et al.* Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. *Eur. Respir. J.* **37**, 1104–1118 (2011).
110. Porvasnik, S. L. *et al.* PRX-08066, a novel 5-hydroxytryptamine receptor 2B antagonist, reduces monocrotaline-induced pulmonary arterial hypertension and right ventricular hypertrophy in rats. *J. Pharmacol. Exp. Ther.* **334**, 364–372 (2010).
111. Mouchaers, K. T. *et al.* Fasudil reduces monocrotaline-induced pulmonary arterial hypertension: comparison with bosentan and sildenafil. *Eur. Respir. J.* **36**, 800–807 (2010).
112. Fujita, H. *et al.* Acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, in patients with pulmonary arterial hypertension. *Heart Vessels* **25**, 144–149 (2010).
113. Fukumoto, Y. *et al.* Acute vasodilator effects of a Rho-kinase inhibitor, fasudil, in patients with severe pulmonary hypertension. *Heart* **91**, 391–392 (2005).
114. Guilluy, C. *et al.* RhoA and Rho kinase activation in human pulmonary hypertension: role of 5-HT signaling. *Am. J. Resp. Crit. Care Med.* **179**, 1151–1158 (2009).
115. Eddahibi, S. *et al.* Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J. Clin. Invest.* **108**, 1141–1150 (2001).
116. Kato, T., Hashikabe, H., Iwata, C., Akimoto, K. & Hattori, Y. Statin blocks Rho/Rho-kinase signaling and disrupts the actin cytoskeleton: relationship to enhancement of LPS-mediated nitric oxide synthesis in vascular smooth muscle cells. *Biochim. Biophys. Acta* **1689**, 267–272 (2004).
117. Nishimura, T. *et al.* Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells. *Circulation* **108**, 1640–1645 (2003).
118. Sun, X. & Ku, D. D. Rosuvastatin provides pleiotropic protection against pulmonary hypertension, right ventricular hypertrophy, and coronary endothelial dysfunction in rats. *Am. J. Physiol. Heart Circ. Physiol.* **294**, H801–H809 (2008).
119. Wilkins, M. R. *et al.* Simvastatin as a treatment for pulmonary hypertension trial. *Am. J. Respir. Crit. Care Med.* **181**, 1106–1113 (2010).
120. Kawut, S. M. *et al.* Randomized clinical trial of aspirin and simvastatin for pulmonary arterial hypertension: ASA-STAT. *Circulation* **123**, 2985–2993 (2011).

121. Zhao, Y. D. *et al.* Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ. Res.* **96**, 442–450 (2005).
122. Wang, X. X. *et al.* Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J. Am. Coll. Cardiol.* **49**, 1566–1571 (2007).
123. US NIH. ClinicalTrials.gov. Pulmonary hypertension: assessment of cell therapy (PHACeT) [online], http://clinicaltrials.gov/ct2/show/NCT00469027?term=PHACeT&rank=1 (2010).
124. Mereles, D. *et al.* Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. *Circulation* **114**, 1482–1489 (2006).
125. Provencher, S. *et al.* Deleterious effects of beta-blockers on exercise capacity and hemodynamics in patients with portopulmonary hypertension. *Gastroenterology* **130**, 120–126 (2006).
126. Mohr, L. C. Hypoxia during air travel in adults with pulmonary disease. *Am. J. Med. Sci.* **335**, 71–79 (2008).
127. Chaouat, A., Weitzenblum, E. & Higenbottam, T. The role of thrombosis in severe pulmonary hypertension. *Eur. Respir. J.* **9**, 356–363 (1996).
128. Fuster, V. *et al.* Primary pulmonary hypertension: natural history and the importance of thrombosis. *Circulation* **70**, 580–587 (1984).
129. Frank, H. *et al.* The effect of anticoagulant therapy in primary and anorectic drug-induced pulmonary hypertension. *Chest* **112**, 714–721 (1997).
130. Bonnin, M. *et al.* Severe pulmonary hypertension during pregnancy: mode of delivery and anesthetic management of 15 consecutive cases. *Anesthesiology* **102**, 1133–1137 (2005).
131. Sitbon, O. *et al.* Long-term response to calcium-channel blockers in idiopathic pulmonary arterial hypertension. *Circulation* **111**, 3105–3111 (2005).
132. Chapelier, A. *et al.* Comparative outcome of heart-lung and lung transplantation for pulmonary hypertension. *J. Thorac. Cardiovasc. Surg.* **106**, 299–307 (1993).
133. Kurzyna, M. *et al.* Atrial septostomy in treatment of end-stage right heart failure in patients with pulmonary hypertension. *Chest* **131**, 977–983 (2007).

Author contributions
D. S. O'Callaghan, L. Savale, and M. Humbert researched data for the article. D. S. O'Callaghan, L. Savale, D. Montani, X. Jaïs, G. Simonneau, and M. Humbert contributed to the discussion of content. The article was written by D. S. O'Callaghan. All authors reviewed and edited the manuscript before submission.