Novel approaches to the pharmacotherapy of pulmonary arterial hypertension

Karen M. Olsson and Marius M. Hoeper

Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany

Pulmonary arterial hypertension (PAH) has evolved from an untreatable condition to a disease for which several classes of drugs have now been approved, including various prostanoids, endothelin receptor antagonists and phosphodiesterase-5 inhibitors. Because the pathogenesis of pulmonary hypertension is increasingly understood, various new substances are now under clinical investigation, including serotonin antagonists, vasoactive intestinal peptide, stimulators of soluble guanylate cyclase and tyrosine kinase inhibitors. Several of these compounds hold promise for the future therapy of PAH, especially as regression of pulmonary vascular remodeling appears to become a realistic possibility with the combination of established and novel treatments.

Introduction

The term pulmonary hypertension describes a condition with elevated pulmonary arterial pressures, that is mean pulmonary artery pressures >20 mmHg at rest. The current clinical classification distinguishes between five major groups of pulmonary hypertension: (i) pulmonary arterial hypertension (PAH), (ii) pulmonary hypertension owing to left heart disease, (iii) pulmonary hypertension owing to lung disease, (iv) chronic thromboembolic pulmonary hypertension and (v) miscellaneous forms [1]. The present article will focus entirely on PAH, a rare but severe form of pulmonary hypertension characterized by progressive pulmonary vascular obliteration, eventually resulting in death from right heart failure.

The etiology of PAH remains incompletely understood, but progress has been made in understanding the pathogenesis of the disease, which is characterized by pulmonary vascular remodeling because of proliferation of endothelial cells and vascular smooth muscle cells, alterations in the extracellular matrix, thrombosis and fibrosis [2]. To provide beneficial long-term effects, treatments must affect pulmonary vascular remodeling at least by slowing disease progression, but ideally by inducing 'reverse remodeling' of the small pulmonary arteries.

Corresponding author: Hoeper, M.M. (hoeper.marius@mh-hannover.de)

This review is intended to provide a brief summary on the existing and emerging treatments for PAH. As a short review article, this paper cannot provide an in-depth overview of this rapidly expanding field so that references will be provided for further reading where available.

Prostanoids and prostacyclin receptor agonists

The prostacyclin analog, epoprostenol was the first drug that showed efficacy in PAH and remains the only drug for which a survival benefit has been proven, at least in functional class IV patients [3]. Long-term survival with intravenous epoprostenol treatment, however, remains limited. The two largest case series have reported three-year survival rates of 63% with this therapy [4,5]. A major drawback of epoprostenol is the need for continuous intravenous administration, because the compound is unstable with a half-life of only a few minutes in aqueous solution.

The prostacyclin analogs, iloprost and treprostinil are also available for continuous intravenous administration [6]. Potential advantages of both compounds compared to epoprostenol are higher chemical stability and longer half-lives which may reduce some of the inconvenience and risk associated with epoprostenol therapy, but there are only preliminary data to show whether all three drugs have comparable long-term efficacy [7,8].

To circumvent the problems associated with continuous intravenous administration, various prostanoids have been developed for subcutaneous, inhaled and oral administration. The stable

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TABLE 1

Mean changes from baseline in hemodynamic variables after three months of therapy with various drugs as shown in randomized, placebo-controlled trials[a].

	Inhaled Iloprost[b] (6 × 5 μg)	Beraprost (4 × 80 μg)	SC Treprostinil (9.3 ng/(kg min))	Bosentan (125 mg bid)	Sitaxentan (100 mg qd)	Sildenafil (20 mg tid)
Refs	[10]	[14]	[65]	[20]	[22]	[34]
PAPm (mmHg)	−0.1	−1	−2.3[c]	−1.6[c]	−3	−2.1[c]
CI (l/(min m^2))	+0.03[d]	+0.2	+0.12[c]	+0.5[c]	+0.3[c]	+0.2
PVR (dynes)	−9[c]	−104	−160[c,e]	−223[c]	−221[c]	−122[c]

The indicated dosages are the mean doses administered at week 12. SC, subcutaneous; PAPm, mean pulmonary artery pressure; CI, cardiac index; PVR, pulmonary vascular resistance.

[a] No hemodynamic data from randomized, placebo-controlled trials are available for epoprostenol and ambrisentan; for comparison, in randomized, nonplacebo-controlled studies, the changes in PVR from baseline after 12 weeks of treatment were −440 dynes with epoprostenol [3] and −277 dynes with ambrisentan [25].
[b] Values measured at trough, that is before inhalation.
[c] Significantly better compared to the placebo group.
[d] In the original paper, numbers were given for cardiac output, which improved by 0.05 l/min; for the purpose of comparability, this value was divided by an assumed value of 1.75 to calculate cardiac index.
[e] In the original paper, numbers were given for pulmonary vascular resistance index, which improved by 280 dynes; for the purpose of comparability, this value was divided by an assumed value of 1.75 to calculate cardiac index.

prostacyclin analog, treprostinil has been approved for subcutaneous administration. The largest series published so far with this treatment ($n = 860$) reported survival rates similar to intravenous prostacyclin [9]. Of the patients who discontinued this therapy prematurely (59%), almost half of them withdrew on account of clinical deterioration (including death) and more than one-third because of side-effects, mostly infusion site pain, which was described by 92% of the patients.

Prostanoids available for inhalation are iloprost and treprostinil. Both compounds have been shown to improve 6 min walk distance in randomized, placebo-controlled trials, but only the trial AIR-1, which studied inhaled iloprost has been fully published [10]. Despite the significant improvement in exercise capacity, hemodynamic improvement in the iloprost group was modest (Table 1). A main problem with inhaled prostanoids is their relatively short duration of action. The hemodynamic effects of iloprost last 30–45 min after each inhalation and patients need to inhale six to nine times per day. Still, the cumulative effective drug exposure adds up to no longer than four to six hours, so large parts of the day may not be fully covered. The hemodynamic effects of inhaled treprostinil last somewhat longer (60–120 min) [11,12], but this advantage may be offset by the strategy of inhaling treprostinil only four times per day. So far, it has not been convincingly shown that the clinical effects of inhaled prostanoids are sustained over longer periods. For inhaled iloprost, the only long-term study published so far showed treatment failure in more than two-thirds of the patients within two years [13].

Two prostanoids are available for oral administration: beraprost and treprostinil. Beraprost has been studied in two randomized, placebo-controlled trials. The first study (ALPHABET) showed a modest effect on 6 min walk distance but no changes in hemodynamics after 12 weeks compared to placebo (Table 1) [14]. The second study addressed long-term efficacy with beraprost therapy. This study showed improved 6 min walk distances compared to placebo after 3 and 6 months of therapy, but this effect was no longer significant at months 9 and 12 [15]. Beraprost has only been approved in Japan and Korea, where an extended-release formulation of the drug recently became available [16]. As with the inhaled prostanoids, there are also no robust long-term data with beraprost, and the new extended-release formulation has not been sufficiently evaluated. Oral treprostinil is currently being studied in an ongoing phase III trial (FREEDOM).

The main problem with oral prostanoids so far has been tolerability, because these drugs often cause unpleasant side-effects, especially headache, jaw pain, gastrointestinal discomfort and diarrhea, often limiting the maximum achievable dose. A new substance currently under investigation is NS-304, an orally available nonprostanoid prostacyclin receptor agonist. NS-304 is a prodrug of the active compound MRE-269 which has a half-life of approximately ten hours. Because NS-304 itself has no activity on the prostanoids receptor, it is expected to have fewer systemic side-effects than orally administered prostanoids. So far, there are no data in terms of the safety and efficacy of NS-304 in PAH, because the first clinical trial with this compound is still underway.

Endothelin receptor antagonists (ERAs)

Endothelin-1 (ET-1) is a potent vasoconstrictor and mitogen for vascular smooth muscle cells that is overexpressed in the pulmonary vasculature of patients with PAH [17]. ET-1 is predominantly released by endothelial cells and acts mostly in a paracrine fashion on two different types of receptors [18]. The ET_A receptor is located on smooth muscle cells and mediates vasoconstrictive, as well as proliferative, signals. ET_B receptors are also located on smooth muscle cells where they probably mediate similar signals like ET_A receptors. ET_B receptors are, however, also expressed on endothelial cells, where they have a clearance function for ET-1 released into the bloodstream and mediate vasodilator signals via liberation of nitric oxide (NO) and prostacyclin [19].

At present, three different ERAs have been studied in and approved for PAH: the ET_A/ET_B antagonist bosentan and the selective ET_A antagonists sitaxentan and ambrisentan. All three compounds improve hemodynamics and exercise capacity in randomized controlled trials of relatively short-term duration, the maximum study time being 18 weeks [20–24]. There are insufficient data to decide whether any of these drugs has superior efficacy compared to the others. The hemodynamic effects of all the three ERA appear to be similar because the reported changes in pulmonary vascular resistance after three months of treatment

with bosentan, sitaxentan and ambrisentan were −223 dynes, −221 dynes and −226 from baseline, respectively [20,22,24]. In addition, the effects on 6 min walking distance were also similar with placebo-corrected improvements of 35 m with bosentan at 125 mg bid and 31 m with sitaxentan at 100 mg qd [21,23]. With ambrisentan, the results are less clear because there were two pivotal studies, ARIES-1 and ARIES-2 [25], which provided somewhat different results. In ARIES-1, 6 min walk distance improved by 31 m with ambrisentan at a dose of 5 mg qd whereas the same dose achieved a 59 m improvement in ARIES-2. Because study design and inclusion/exclusion criteria were virtually identical in both studies, the reasons for this difference are not clear. The data on long-term survival with any of these drugs, with the exception of bosentan, is limited to the long-term extensions of the pivotal trials and one-year survival rates were in the range of 95% with all three drugs [19]. These data, however, must be interpreted with caution, not only because it is difficult to compare different trials, but also because patients entering those trials are preselected because they have to fulfill criteria of stability to be eligible for a placebo-controlled clinical trial.

Until recently, ERA were approved only for PAH in functional class III (Europe), or III and IV (United States). Ambrisentan has now also been approved for functional class II based on a significant improvement in 6 min walk distance in this subgroup in the ARIES studies. Important data showing that ERA are efficacious in class II patients came also from the EARLY trial [26]. This study addressed a well-characterized population of PAH patients in functional class II who were randomized to receive either bosentan or placebo in a double-blind fashion for six months. The EARLY trial had two coprimary endpoints: change in pulmonary vascular resistance (PVR) and 6 min walk distance. At the end of the study period there was a significant improvement in PVR in bosentan-treated patients, whereas the change in 6 min walk distance did not reach statistical significance. Time to clinical worsening, a secondary composite endpoint including death, transplantation and predefined indicators of clinical deterioration was, however, significantly improved in the bosentan group with a relative risk reduction of 77%. This study not only demonstrated that it is useful to treat PAH patients in functional class II but, perhaps more importantly, also showed for the first time that targeted treatment can prevent clinical deterioration in PAH patients, even when exercise capacity is not improved.

There are differences in the safety profiles of the three available ERA. Potential liver toxicity is being regarded as a class effect of ERA, but the long-term extension data from the pivotal trials suggest that aminotransferase elevations occur more commonly with bosentan (11.6%) than with sitaxentan (7%) or ambrisentan (2%). So far there is no evidence, however, linking bosentan or any other ERA at currently approved dosages to permanent liver damage or liver failure [27], but long-term data are not available for sitaxentan and ambrisentan. Nevertheless, liver enzymes need to be monitored on a monthly basis with all three drugs. In addition to hepatotoxicity, ERA have been linked to edema formation although the pathophysiological mechanisms are poorly understood. So far, it is unclear whether there are differences between the three ERA in terms of incidence or severity of edema formation [19].

Pharmacokinetic interactions might also be relevant when ERA are used together with other compounds. As far as typical comedications in PAH patients are concerned, interactions with warfarin and sildenafil are of particular importance. Bosentan induces cytochrome enzymes and augments the metabolism of warfarin and sildenafil [28,29]. Sitaxentan, an inhibitor of cytochromes, has no pharmacokinetic interactions with sildenafil, but reduces the metabolism of warfarin. Ambrisentan has apparently no clinically relevant drug–drug interactions [19].

Macitentan, a novel ET_A/ET_B receptor antagonist is currently being studied in a phase III trial in PAH (SERAPHIN).

Phosphodiesterase (PDE) inhibitors

Several PDEs are upregulated in the pulmonary vessels of PAH patients [30,31]. The isoenzyme PDE-5 has gained particular attention after clinical observations suggested beneficial effects of the PDE-5 inhibitor sildenafil in PAH patients [32]. PDE-5 deactivates cyclic GMP (cGMP), the second messenger mediating the effects of NO and other pulmonary vasodilators [33].

SUPER-1, the pivotal trial studying the effects of sildenafil in PAH compared three dosages of sildenafil: 20 mg, 40 mg and 80 mg, three times a day with placebo [34]. After 12 weeks, the placebo-corrected improvements were similar to all three dosages, ranging from +45 m to +50 m. On the basis of these results, sildenafil has been approved in many countries at a dose of 20 mg tid. SUPER-1 indicated, however, that the hemodynamic effects of sildenafil were dose-dependent and the highest dose of 80 mg tid had the strongest effect on PVR (−310 dyn versus −171 dyn and −192 dyn with 20 mg and 40 mg tid). All dosages were equally well tolerated and the open-label extension of the study had a target dose of 80 mg tid (unpublished data). For the time being, there are virtually no long-term data on the 20 mg tid dosage and many experts believe that higher dosages might be more efficacious in the long run [35].

Tadalafil, a long-acting PDE-5 inhibitor is also being studied in PAH. A phase III study has recently been concluded, but results have not yet been published.

Direct comparisons between sildenafil and tadalafil are not yet available, except for acute hemodynamic data [36]. The longer duration of action is a potential advantage of tadalafil, but it is unclear whether both drugs are going to have the same long-term effects on disease progression. Of note, sildenafil is not a truly selective PDE-5 inhibitor, because it also blocks the PDE-1 isoenzyme. This effect may be of particular interest because PDE-1 is involved in vascular smooth muscle cell proliferation [37]. Tadalafil, by contrast, has no effect on PDE-1. It is not clear if these considerations are of clinical relevance, but head-to-head comparisons between sildenafil and tadalafil addressing long-term safety and efficacy in PAH are desirable.

Stimulators and activators of soluble guanylate cyclase

The soluble guanylate cyclase (sGC) is an attractive target for treating PAH [38,39]. As shown in Fig. 1, the NO-induced cGMP-activation is mediated via sGC, a heme-iron (II)-containing enzyme. In PAH, sGC is upregulated in the pulmonary arteries but at the same time the enzyme may become inactivated by the oxidation of the heme-iron [40]. In that case, NO and other sGC-dependent vasodilators such as the natriuretic peptides lose

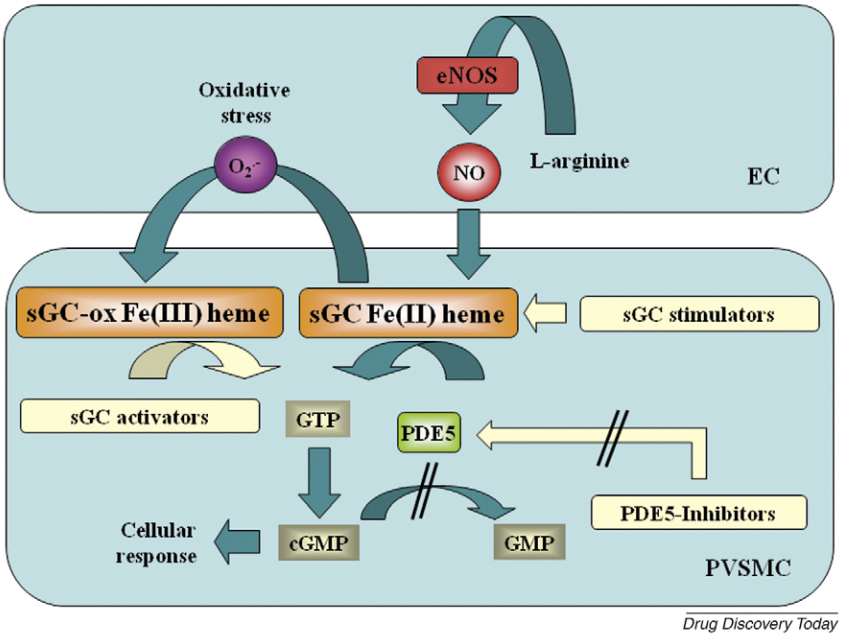

FIGURE 1

Pharmacological targets of the nitric oxide (NO)–soluble guanylate cyclase (sGC)–cyclic GMP (cGMP) system. In the physiological state, NO activates sGC upon binding to Fe(II) heme. Under conditions of oxidative stress oxygen radicals ($O_2^{\bullet-}$) inactivate sGC by reducing the heme-iron (Fe(III) heme). sGC stimulators and activators increase cGMP production, sGC stimulators by acting preferentially on the nonoxidized Fe(II) heme, sGC activators by acting preferentially on the Fe(III) heme. On the basis of their actions, sGC stimulators require the presence of NO whereas sGC activators act independently of NO. Phosphodiesterase-5 inhibitors augment intracellular cGMP by blocking its degradation. EC, endothelial cell; PVSMC, pulmonary vascular smooth muscle cell; GTP, guanosine triphosphate; eNOS, endothelial NO synthase.

their vasodilatory potential. Two different compounds are now being studied in pulmonary vascular disease, sGC stimulators and sGC activators. The stimulators augment the NO effects on the enzyme, whereas the activators act preferably on the oxidized (iron-III) heme, thus having the potential to induce vasodilation in the absence of NO. Both sGC activators and stimulators partly reverse pulmonary vascular remodeling in experimental models of pulmonary hypertension [40]. Acute hemodynamic studies in 10 PAH patients showed profound hemodynamic effects of BAY 63-2521, an orally available sGC stimulator, with a reduction in PVR of more than 30% from baseline when given as a single dose of 2.5 mg (unpublished data). BAY 63-2521 caused significant reductions not only in pulmonary artery pressures, but also in systemic artery pressures accompanied by a marked improvement in cardiac index (unpublished data). The systemic hypotensive effect, however, seems to abate with long-term administration. A phase II trial with BAY 63-2521 in patients with PAH and chronic thromboembolic pulmonary hypertension (CTEPH) was recently concluded, but the results are not yet available. This compound will now be studied in phase III in PAH and CTEPH as well as in phase II in patients with pulmonary hypertension associated with interstitial lung disease (ILD).

Statins

In animal models of pulmonary hypertension, high dosages of simvastatin are capable of reversing pulmonary vascular remodeling [41] and pilot clinical trials are ongoing to investigate whether statins have beneficial effects in PAH. On the basis of clinical experience, it is unlikely that statins are going to improve hemodynamics and exercise capacity in PAH patients and the most important question to be answered is whether statins have the potential to slow disease progression.

Serotonin (5-HT) receptor antagonists and serotonin transporter (5-HTT) blockers

For many years, the serotonin system has been implicated in the pathophysiology of pulmonary hypertension [42–44]. A detailed review of the biology of the serotonin system in vascular disease is beyond the scope of this review article. At the present time, several serotonin receptor antagonists are being investigated for PAH but none of these trials have been concluded.

Rho-kinase inhibitors

The Rho/Rho-kinase system plays an important role in the regulation of vascular tone and vascular smooth cell proliferation and may, therefore, be an attractive target for pharmacological intervention [45]. Fasudil, a Rho-kinase inhibitor has not only shown promising effects in animal models of pulmonary hypertension [46,47], but has also been associated with renal toxicity. Currently it is unclear whether fasudil will be further evaluated in PAH.

Vasoactive intestinal peptide (VIP)

VIP is a peptide neurotransmitter with potent vasodilatory properties and a deficiency of VIP has been demonstrated in serum as well as in lung tissue, of patients with PAH [48]. A single pilot study with inhaled VIP performed several years ago reported impressive

effects on hemodynamics and exercise capacity at a dose of 4×50 μg per day [48]. Only recently, another group of investigators evaluated the acute hemodynamic effects of inhaled synthetic VIP (aviptadil) at a dose of 100 μg and the observed changes in pulmonary artery pressure and pulmonary vascular resistance were modest, at best, certainly far less than in the original paper [49]. However, VIP may affect pulmonary vascular remodeling independently of any vasodilator activity [50,51]. Currently, a clinical trial with inhaled aviptadil is ongoing that is designed to identify the most effective dose of VIP, as well as to provide preliminary data on safety and efficacy.

Tyrosine kinase inhibitors (TKIs)

TKIs are among the most promising new agents in the PAH field, because they, for the first time, offer a realistic option of achieving so-called 'reverse-remodeling' of the pulmonary vessels. Imatinib is a TKI that has been originally developed to block the BCR-ABL tyrosine kinase responsible for the development of chronic myelogenous leukemia. Imatinib, however, also inhibits the tyrosine kinase of the platelet-derived growth factor receptor, which is involved in the proliferation of vascular smooth muscle cells. In experimental models of pulmonary hypertension, imatinib is capable of reversing fully established media hypertrophy, presumably by causing apoptosis of vascular smooth muscle cells [52]. A few case reports suggest that imatinib can have impressive clinical and hemodynamic effects in patients with advanced PAH [53–55]. Because imatinib is not a pulmonary vasodilator, it appears that the drug may indeed affect pulmonary vascular remodeling. It is already clear that not all PAH patients are responsive to imatinib treatment and much more research is needed to clarify if this drug is a useful adjunct to PAH therapy, especially because the safety of imatinib in PAH patients is unknown. A phase II randomized, placebo-controlled trial with imatinib in patients with severe PAH was concluded in April 2008, but results await publication. Other TKIs with different receptor targeting profiles and multikinase inhibitors [56] are also now being studied for PAH.

Combination therapy

Because all of the currently available treatments show modest hemodynamic improvement (Table 1) and limited long-term efficacy, combination of compounds that act via different intracellular mechanisms is a logical approach. In fact, combination therapy has become the standard of care in many pulmonary hypertension centers throughout the world [57–61] although the evidence to support this strategy remains limited. So far, only few randomized controlled trials have addressed combination therapy in PAH. STEP-1 was a randomized, placebo-controlled trial that studied the addition of inhaled iloprost to bosentan [62]. After 12 weeks of therapy the change in 6 min walk distance was of borderline significance compared to placebo (+26 m, $P = 0.051$), but hemodynamics remained virtually unchanged. There was, however, a significant improvement in time to clinical worsening (five events in the placebo group versus none in the iloprost group; $P = 0.022$). At the same time, COMBI, another randomized trial studying the effects of adding inhaled iloprost to bosentan, showed no improvements in exercise capacity or time to clinical worsening [63]. Thus, the strategy of adding inhaled iloprost to bosentan needs further evaluation, especially in terms of long-term efficacy.

The only other randomized controlled trial addressing combination therapy that has been concluded was PACES, a study testing the addition of sildenafil to epoprostenol over a 16-week period. This trial, which included 267 patients, showed that the combination of epoprostenol and sildenafil had significantly better effects on exercise capacity and time to clinical worsening than epoprostenol alone [64]. Of note, there were seven deaths in this study, all of them in the monotherapy group. These data strongly suggest that combination therapy is indeed having an effect on the outcome of patients with PAH.

Combining ERA and PDE-5 inhibitors is particularly appealing, although the evidence supporting this concept is, so far, limited to some case series reporting beneficial clinical effects when sildenafil was added to bosentan [58,61]. COMPASS-2 is an ongoing randomized placebo-controlled study studying the combination of sildenafil and bosentan versus sildenafil monotherapy. This will be the first randomized, controlled study to look at long-term morbidity and mortality in the field of PAH, but results are not expected before 2010. In addition, other trials are currently planned to address the long-term effects of the upfront use of combination therapy with ERA and PDE-5 inhibitors in PAH.

Outlook

The therapeutic options for PAH are rapidly evolving: currently the number of potential new treatments and clinical trials seems to surpass the number of patients available for clinical trials. It is important that all new treatments are studied carefully and that future trials focus on long-term outcome, rather than on short-term improvements in exercise capacity. This is particularly relevant for combination therapy, not at least given the enormous costs of PAH medications. In addition, the non-PAH forms of pulmonary hypertension need to be studied carefully to find out if other groups of patients can also benefit from targeted medical therapy.

Conflict of interest statement

KMO has received speaker fees from Bayer/Schering. MMH has received fees for consultations and/or speaking at conferences from Actelion Pharmaceuticals, Bayer, Encysive Pharmaceuticals, GSK, Novartis, LungRx and Pfizer.

References

1 Simonneau, G. *et al.* (2004) Clinical classification of pulmonary hypertension. *J. Am. Coll. Cardiol.* 43 (Suppl. S), 5S–12S

2 Farber, H.W. and Loscalzo, J. (2004) Pulmonary arterial hypertension. *N. Engl. J. Med.* 351, 1655–1665

3 Barst, R.J. *et al.* (1996) 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

4 Sitbon, O. *et al.* (2002) Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. *J. Am. Coll. Cardiol.* 40, 780–788
5 McLaughlin, V.V. *et al.* (2002) Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. *Circulation* 106, 1477–1482
6 Olschewski, H. and Gomberg-Maitland, M. (2008) Prostacyclin therapies for the treatment of pulmonary arterial hypertension. *Eur. Respir. J.* 31, 801–901
7 Higenbottam, T. *et al.* (1998) Long-term intravenous prostaglandin (epoprostenol or iloprost) for treatment of severe pulmonary hypertension. *Heart* 80, 151–155
8 Gomberg-Maitland, M. *et al.* (2005) Transition from intravenous epoprostenol to intravenous treprostinil in pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 172, 1586–1589
9 Barst, R.J. *et al.* (2006) Long-term outcome in pulmonary arterial hypertension patients treated with subcutaneous treprostinil. *Eur. Respir. J.* 28, 1195–1203
10 Olschewski, H. *et al.* (2002) Inhaled iloprost for severe pulmonary hypertension. *N. Engl. J. Med.* 347, 322–329
11 Voswinckel, R. *et al.* (2006) Inhaled trepostinil for treatment of chronic pulmonary arterial hypertension. *Ann. Intern. Med.* 144, 149–150
12 Voswinckel, R. *et al.* (2006) Favorable effects of inhaled treprostinil in severe pulmonary hypertension: results from randomized controlled pilot studies. *J. Am. Coll. Cardiol.* 48, 1672–1681
13 Opitz, C.F. *et al.* (2005) Clinical efficacy and survival with first-line inhaled iloprost therapy in patients with idiopathic pulmonary arterial hypertension. *Eur. Heart J.* 26, 1895–1902
14 Galie, N. *et al.* (2002) Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Cardiol.* 39, 1496–1502
15 Barst, R.J. *et al.* (2003) Beraprost therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 41, 2119–2125
16 Ikeda, D. *et al.* (2007) Pilot study of short-term effects of a novel long-acting oral beraprost in patients with pulmonary arterial hypertension. *Circ. J.* 71, 1829–1831
17 Giaid, A. *et al.* (1993) Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.* 328, 1732–1739
18 Luscher, T.F. and Barton, M. (2000) Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. *Circulation* 102, 2434–2440
19 Dupuis, J. and Hoeper, M.M. (2008) Endothelin receptor antagonists in pulmonary arterial hypertension. *Eur. Respir. J.* 31, 407–414
20 Channick, R.N. *et al.* (2001) Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet* 358, 1119–1123
21 Rubin, L.J. *et al.* (2002) Bosentan therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* 346, 896–903
22 Barst, R.J. *et al.* (2004) Sitaxsentan therapy for pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 169, 441–447
23 Barst, R.J. *et al.* (2006) Treatment of pulmonary arterial hypertension with the selective endothelin-A receptor antagonist sitaxsentan. *J. Am. Coll. Cardiol.* 47, 2049–2056
24 Galie, N. *et al.* (2008) 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
25 Galie, N. *et al.* (2005) Ambrisentan therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 46, 529–535
26 Galie, N. *et al.* (2008) Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *Lancet* 371, 2093–2100
27 Humbert, M. *et al.* (2007) Results of European post-marketing surveillance of bosentan in pulmonary hypertension. *Eur. Respir. J.* 30, 338–344
28 Murphey, L.M. and Hood, E.H. (2003) Bosentan and warfarin interaction. *Ann. Pharmacother.* 37, 1028–1031
29 Paul, G.A. *et al.* (2005) Bosentan decreases the plasma concentration of sildenafil when coprescribed in pulmonary hypertension. *Br. J. Clin. Pharmacol.* 60, 107–112
30 Dony, E. *et al.* (2008) Partial reversal of experimental pulmonary hypertension by phosphodiesterase-3/4 inhibition. *Eur. Respir. J.* 31, 599–610
31 Wharton, J. *et al.* (2005) Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. *Am. J. Respir. Crit. Care Med.* 172, 105–113
32 Prasad, S. *et al.* (2000) Sildenafil in primary pulmonary hypertension. *N. Engl. J. Med.* 343, 1342
33 Wilkins, M.R. *et al.* (2008) Phosphodiesterase inhibitors for the treatment of pulmonary hypertension. *Eur. Respir. J.* 32, 198–209
34 Galie, N. *et al.* (2005) Sildenafil citrate therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* 353, 2148–2157
35 Hoeper, M.M. and Welte, T. (2006) Sildenafil citrate therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* 354, 1091–1093 author reply 1091–1093
36 Ghofrani, H.A. *et al.* (2004) Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension: a randomized prospective study. *J. Am. Coll. Cardiol.* 44, 1488–1496
37 Schermuly, R.T. *et al.* (2007) Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy. *Circulation* 115, 2331–2339
38 Stasch, J.P. *et al.* (2001) NO-independent regulatory site on soluble guanylate cyclase. *Nature* 410, 212–215
39 Frey, R. *et al.* (2008) Single-dose pharmacokinetics, pharmacodynamics, tolerability, and safety of the soluble guanylate cyclase stimulator BAY 63-2521: an ascending-dose study in healthy male volunteers. *J. Clin. Pharmacol.* 48 (8), 926–934
40 Schermuly, R.T. *et al.* (2008) Expression and function of soluble guanylate cyclase in pulmonary arterial hypertension. *Eur. Respir. J.* 32, 881–891
41 Nishimura, T. *et al.* (2003) Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells. *Circulation* 108, 1640–1645
42 Eddahibi, S. *et al.* (2006) Cross talk between endothelial and smooth muscle cells in pulmonary hypertension: critical role for serotonin-induced smooth muscle hyperplasia. *Circulation* 113, 1857–1864
43 Eddahibi, S. *et al.* (2001) Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J. Clin. Invest.* 108, 1141–1150
44 Guignabert, C. *et al.* (2005) Serotonin transporter inhibition prevents and reverses monocrotaline-induced pulmonary hypertension in rats. *Circulation* 111, 2812–2819
45 Hirooka, Y. and Shimokawa, H. (2005) Therapeutic potential of rho-kinase inhibitors in cardiovascular diseases. *Am. J. Cardiovasc. Drugs* 5, 31–39
46 Jiang, B.H. *et al.* (2007) Acute vasodilator effect of fasudil, a rho-kinase inhibitor, in monocrotaline-induced pulmonary hypertension in rats. *J. Cardiovasc. Pharmacol.* 49, 85–89
47 Nagaoka, T. *et al.* (2005) Inhaled rho kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 171, 494–499
48 Petkov, V. *et al.* (2003) Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J. Clin. Invest.* 111, 1339–1346
49 Leuchte, H.H. *et al.* (2008) Inhalation of vasoactive intestinal peptide in pulmonary hypertension. *Eur. Respir. J.* 32, 1289–1294
50 Hamidi, S.A. *et al.* (2008) Enhancement of pulmonary vascular remodelling and inflammatory genes with VIP gene deletion. *Eur. Respir. J.* 31, 135–139
51 Said, S.I. *et al.* (2007) Moderate pulmonary arterial hypertension in male mice lacking the vasoactive intestinal peptide gene. *Circulation* 115, 1260–1268
52 Schermuly, R.T. *et al.* (2005) Reversal of experimental pulmonary hypertension by PDGF inhibition. *J. Clin. Invest.* 115, 2811–2821
53 Ghofrani, H.A. *et al.* (2005) Imatinib for the treatment of pulmonary arterial hypertension. *N. Engl. J. Med.* 353, 1412–1413
54 Patterson, K.C. *et al.* (2006) Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension. *Ann. Intern. Med.* 145, 152–153
55 Souza, R. *et al.* (2006) Long term imatinib treatment in pulmonary arterial hypertension. *Thorax* 61, 736
56 Klein, M. *et al.* (2008) Combined tyrosine and serine/threonine kinase inhibition by sorafenib prevents progression of experimental pulmonary hypertension and myocardial remodeling. *Circulation* 118, 2081–2090
57 Hoeper, M.M. *et al.* (2005) Goal-oriented treatment and combination therapy for pulmonary arterial hypertension. *Eur. Respir. J.* 26, 858–863
58 Hoeper, M.M. *et al.* (2004) Combination therapy with bosentan and sildenafil in idiopathic pulmonary arterial hypertension. *Eur. Respir. J.* 24, 1007–1010
59 Hoeper, M.M. *et al.* (2003) Bosentan treatment in patients with primary pulmonary hypertension receiving nonparenteral prostanoids. *Eur. Respir. J.* 22, 330–334
60 Ghofrani, H.A. *et al.* (2003) Oral sildenafil as long-term adjunct therapy to inhaled iloprost in severe pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 42, 158–164
61 Mathai, S.C. *et al.* (2007) Addition of sildenafil to bosentan monotherapy in pulmonary arterial hypertension. *Eur. Respir. J.* 29, 469–475

62 McLaughlin, V.V. *et al.* (2006) Randomized study of adding inhaled iloprost to existing bosentan in pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 174, 1257–1263
63 Hoeper, M.M. *et al.* (2006) Combining inhaled iloprost with bosentan in patients with idiopathic pulmonary arterial hypertension. *Eur. Respir. J.* 28, 691–694
64 Simonneau, G. *et al.* (2008) Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension: a randomized trial. *Ann. Intern. Med.* 149, 521–530
65 Simonneau, G. *et al.* (2002) Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 165, 800–804