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REVIEW

New perspectives for the treatment of pulmonary hypertension

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Pulmonary hypertension (PH) is a debilitating disease with a poor prognosis. Therapeutic options remain limited despite the introduction of prostacyclin analogues, endothelin receptor antagonists and phosphodiesterase 5 inhibitors within the last 15 years; these interventions address predominantly the endothelial and vascular dysfunctionS associated with the condition, but simply delay progression of the disease rather than offer a cure. In an attempt to improve efficacy, emerging approaches have focused on targeting the pro-proliferative phenotype that underpins the pulmonary vascular remodelling in the lung and contributes to the impaired circulation and right heart failure. Many novel targets have been investigated and validated in animal models of PH, including modulation of guanylate cyclases, phosphodiesterases, tyrosine kinases, Rho kinase, bone morphogenetic proteins signalling, 5-HT, peroxisome proliferator activator receptors and ion channels. In addition, there is hope that combinations of such treatments, harnessing and optimizing vasodilator and anti-proliferative properties, will provide a further, possibly synergistic, increase in efficacy; therapies directed at the right heart may also offer an additional benefit. This overview highlights current therapeutic options, promising new therapies, and provides the rationale for a combination approach to treat the disease.

LINKED ARTICLES

This article is part of a themed issue on Respiratory Pharmacology. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-1

Abbreviations

AC, adenylate cyclase; ACE., angiotensin converting enzyme; BH_4, tetrahydrobiopterin; BMPR, bone morphogenetic protein receptor; CCB, calcium channel blockers; CTEPH, chronic thromboembolic PH; eNOS, endothelial NO synthase; EPC, endothelial progenitor cell; ERA, endothelin receptor antagonist; ERK1/2, extra-cellular signal regulated kinase; ET-1, endothelin-1; FGF, fibroblast growth factor; IP, prostacyclin receptor; JAK/STAT, Janus kinase/signal transducer and activator of transcription; K_v, voltage-sensitive potassium channel; MAPK, mitogen-activated protein kinase; NEP, neutral endopeptidase; NP, natriuretic peptide; NPR, natriuretic peptide receptor; PAH, pulmonary arterial hypertension; PDGF, platelet derived growth factor; pGC, particulate guanylate cyclase; PGI_2, prostacyclin; PH, pulmonary hypertension; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; PPAR, peroxisome proliferator activated receptor; PPHN, persistent pulmonary hypertension of the newborn; PVR, pulmonary vascular resistance; ROCK, Rho-associated kinase; SERT, 5-HT transporter; sGC, soluble guanylate cyclase; SNP, single nucleotide polymorphism; Src, Src kinase; SSRI, selective 5-HT reuptake inhibitor; TGFβ, transforming growth factor β; TRK, tyrosine kinase receptor; TRPC6, transient receptor potential C6; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; VOCC, voltage operated calcium channel; XNT, 1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulphonyl oxy]-9H-xanthene-9-one)

Introduction

Pulmonary hypertension (PH) is a multi-factorial, progressive disease with substantial mortality and morbidity. Despite recent improvements in treatment, the mortality associated with PH remains high, with survival at 2 years from diagnosis approximately 85% (Thenappan *et al.*, 2007; National Pulmonary Hypertension Centres of the UK and Ireland, 2008). Unfortunately, there remains no cure and clinical worsening is merely delayed, not prevented, by therapy (McLaughlin *et al.*, 2009a,b). However, advances in the understanding of PH aetiology and pathology have yielded novel concepts, drug targets and treatment strategies that may improve the management of patients with the disease. This overview will provide a brief overview of the current therapeutic options and highlight some of these emerging therapeutic approaches which hold promise for alleviating this debilitating disorder with an extremely poor prognosis.

Pulmonary arterial hypertension

Since the World Health Organization (WHO) oversaw the initial categorization of PH into 'primary' and 'secondary' forms in the early 1970 s, based on the presence or absence of identifiable causes or risk factors, the clinical classification of PH has undergone numerous modifications. The goal of the current organization of PH is to group together different manifestations of the disease, sharing similarities in pathophysiological mechanisms, clinical presentation and therapeutic approaches (Figure 1; Simonneau *et al.*, 2009).

Pulmonary arterial hypertension (PAH) is a subset of pulmonary hypertensive syndromes, defined by a resting mean

1. Pulmonary Arterial Hypertension (PAH)
    - 1.1. Idiopathic (IPAH)
    - 1.2. Heritable/familial (FPAH)
        - 1.2.1. BMPR2
        - 1.2.2. ALK1, Endoglin
        - 1.2.3. Unknown
    - 1.3. Drug and toxin-induced
    - 1.4. Associated with (APAH)
        - 1.4.1.Conective tissue disorders
        - 1.4.2. HIV infection
        - 1.4.3. Portal hypertension
        - 1.4.4. Congenital heart diseases
        - 1.4.5. Schistosomiasis
        - 1.4.6. Chronic haemolytic anaemia
    - 1.5. Persistent pulmonary hypertension of the newborn (PPHN)

    1'. Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary haemangiomatosis (PCH)

2. Pulmonary hypertension with left heart disease
    - 2.1. Systolic dysfunction
    - 2.2. Diastolic dysfunction
    - 2.3. Valvular disease

3. Pulmonary hypertension due to lung diseases and/or hypoxia
    - 3.1. Chronic obstructive pulmonary disease (COPD)
    - 3.2. Interstitial lung disease
    - 3.3. Other pulmonary diseases with mixed restrictive and obstructive pattern
    - 3.4. Sleep disordered breathing
    - 3.5. Alveolar hyperventilation disorders
    - 3.6. Chronic exposure of high altitude
    - 3.7. Developmental abnormalities

4. Chronic thromboembolic pulmonary hypertension (CTEPH)

5. Pulmonary hypertension with indistinct, multi-factorial mechanisms
    - 5.1. Haematological disorders (e.g. myeloproliferative disorders, splenectomy, haemoglobinopathies)
    - 5.2. Systemic disorders (e.g. sarcoidosis, pulmonary Langerhans cell histocytosis , lymphangiomatosis)
    - 5.3. Metabolic disorders (e.g. glycogen storage disease, Gaucher's disease, thyroid disorders)
    - 5.4. Others (e.g. tumoural obstruction, fibrosing mediastinitis, chronic renal failure and dialysis)

Figure 1
Current classification of pulmonary hypertension.

pulmonary artery pressure (PAP) > 25 mm Hg, pulmonary vascular resistance (PVR) > 3 Wood units and pulmonary wedge pressure <15 mmHg, in the absence of other causes of PH (Archer *et al.*, 1998). PAH is primarily a disease of the small pulmonary arteries, characterized by vascular proliferation, remodelling and progressive increases in PVR, leading ultimately to right ventricular failure and death (Voelkel *et al.*, 2006). PVR increases are attributed to endothelial dysfunction, resulting in vasoconstriction, remodelling of the pulmonary vessel wall and thrombosis *in situ* (Budhiraja *et al.*, 2004). However, the haemodynamic aberrations represent only one aspect of PAH, and enhanced proliferation, decreased apoptosis and a shift to glycolytic metabolism in pulmonary artery smooth muscle cell fibroblasts and endothelial cells is now recognized as central to the pathogenesis of the disease. This pro-proliferative phenotype is underpinned, at least in a subset of PAH patients, by aberrations in transforming growth factor (TGF)β signalling. This concept has been brought about by the identification of loss-of-function mutations in the bone morphogenetic protein receptor-2 (BMPR2) gene that promotes cell proliferation and suppress apoptosis in 80% of familial PAH patients (Lane *et al.*, 2000). BMPR2 mutations are, however, uncommon in non-familial PAH (10–20%) and even in familial PAH; disease penetrance is low (only 25% of carriers develop the disease; Newman *et al.*, 2004). Single nucleotide polymorphisms (SNPs) in ion channels (e.g. K_v1.5, TRPC6) and transporter genes (SERT; molecular target nomenclature follows Alexander *et al.*, 2009) can also predispose to PAH and a multi-hit hypothesis, a complex interaction between genes and environment, has been proposed to explain the low disease penetrance of genetic mutations in PAH [Figure 2; (Yuan and Rubin, 2005; Newman *et al.*, 2008)].

Current therapy

To date, clinical evaluation of novel therapies for PH has been confined primarily to the PAH subset; there is only limited randomized clinical trial evidence for other forms of PH, for example, associated with lung disease and chronic thromboembolic PH (CTEPH), and research to establish effectiveness of these therapies across PH classes is needed. Nonetheless, advances made in the treatment of PAH are also likely to be effective, to a greater or lesser extent, in patients with aetiologically distinct forms of PH.

Many PH patients receive a background therapy of warfarin, diuretics, digoxin and oxygen (McLaughlin *et al.*, 2009a,b). Anti-coagulant therapy with warfarin appears to have beneficial effects on survival, at least based on findings of observational studies (Johnson *et al.*, 2006), while diuretics limit oedema, and digoxin and oxygen provide symptomatic relief. Frontline therapy aims at enhancing vasodilatation, predominantly by inhibiting the bioactivity of endothelin-1 (ET-1), a potent endothelium-derived vasoconstrictor, or by augmenting the vasodilator properties of nitric oxide (NO) and prostacyclin (PGI_2).

Ca^{2+} channel blockers

L-type Ca^{2+} channel blockers (CCB), such as. nifedipine, diltiazem or amlodipine, can be effective in patients that respond to a one-time vasodilator challenge with a >20% fall in PAP and no decline in cardiac output (Rich and Brundage, 1987). Notably, only 10–15% of patients with iPAH meet these criteria and only half of those will receive sustained clinical and haemodynamic benefit. Patients who respond to CCB therapy, however, have an excellent 5 year survival rate (94%) as compared with those that do not respond [55% survival; (Sitbon *et al.*, 2005)].

Prostacyclin analogues

PGI_2 and thromboxane A_2 (TXA_2) are arachidonic acid metabolites with opposing vasoactivity. In PAH, the balance is shifted towards vasoconstrictor, pro-proliferative TXA_2 from vasodilator, anti-proliferative PGI_2 (Christman *et al.*, 1992; Tuder *et al.*, 1999). This relative impairment in PGI_2-dependent signalling in PAH leads to the development of analogues that would mimic the cytoprotective activity of this prostanoid and restore the balance between PGI_2 and TXA_2. The beneficial activity of prostacyclin (analogues) in PH is presumed to be via activation of the G_s-coupled IP receptor, despite the fact that these compounds can activate other prostanoid receptors (Narumiya *et al.*, 1999); however, recent evidence also supports a role for peroxisome proliferator activated receptors (PPARs; see below) in the underlying mechanism.

Epoprostenol was the first treatment targeted directly at PAH pathology, and has a proven survival advantage (Rubin *et al.*, 1990; Barst *et al.*, 1996; Badesch *et al.*, 2009). Its poor stability, cost and the need for parenteral infusion, however, have led to the development of more stable analogues with more favourable means of administration and pharmacokinetic profiles; iloprost, trepostinil and beraprost are all used in the clinical management of PAH patients.

Endothelin receptor antagonists (ERAs)

Plasma levels of ET-1, a potent vasoconstrictor and mitogenic agent, are significantly elevated and correlate with disease severity in PAH (Rubens *et al.*, 2001). The action of ET-1 is complex and mediated via two cell-surface, G-protein-coupled receptors; ET_A receptors on vascular smooth muscle cells cause vasoconstriction and proliferation, while ET_B receptors on endothelial cells stimulate NO and prostacyclin release, but on vascular smooth muscle cells induce vasoconstriction and mitogenesis. Endothelin receptor antagonists such as bosentan (dual ET_A/ET_B), ambrisentan ($ET_A > ET_B$) and sitaxsentan ($ET1_A >> ET_B$) have been shown to improve pulmonary haemodynamics, exercise capacity and reduce PAH symptoms (Williamson *et al.*, 2000; Channick *et al.*, 2001; Barst *et al.*, 2004; Galie *et al.*, 2005a); these drugs are of clinical benefit, particularly in PAH associated with connective tissue disease where they are often used as the initial treatment option (Denton *et al.*, 2008). However, a positive survival effect, and relative comparisons between ET-1 receptor selective agents (selective ET_A antagonists should possess a theoretical advantage in not preventing the production of NO via ET_B receptor activation on endothelial cells) are still lacking. Macitentan, a novel ET_A/ET_B receptor antagonist, is currently in a phase III trial in PAH (SERAPHIN), after producing a promising haemodynamic profile in a smaller Phase II trial in hypertensive patients (Raja, 2010).

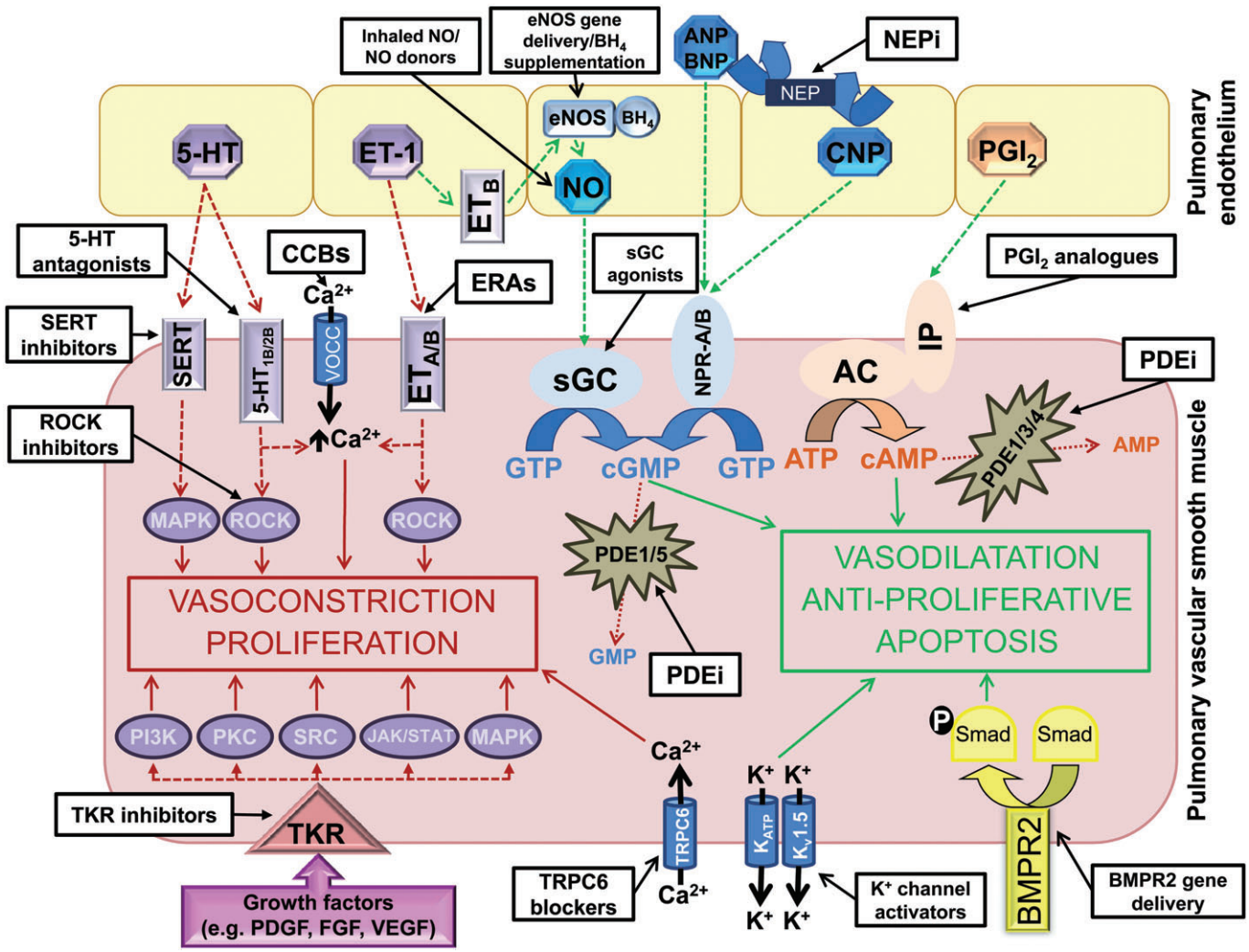

Figure 2

Schematic representation of the current and emerging therapeutic targets for pulmonary hypertension outlined in this review. NP, natriuretic peptide; NPR, natriuretic peptide receptor; PGI_2, prostacyclin; ET-1, endothelin-1; BMPR, bone morphogenetic protein receptor; K_v, voltage-sensitive potassium channel; K_{ATP}, ATP-sensitive potassium channel; TRPC6, transient receptor potential channel C6; VOCC, voltage operated calcium channel; SERT, 5-HT transporter; NEP, neutral endopeptidase; NEPi, NEP inhibitor; BH_4, tetrahydrobiopterin; eNOS, endothelial NO synthase; TKR, tyrosine kinase receptor; ROCK, Rho-associated kinase; sGC, soluble guanylate cyclase; ERA, endothelin receptor antagonist; PDGF, platelet derived growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; AC, adenylate cyclase; IP, prostacyclin receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; Src, Src kinase; JAK/STAT, Janus kinase/signal transducer and activator of transcription; PDE, phosphodiesterase; PDEi, PDE inhibitor.

PDE5 inhibitors

PDEs are homologous enzymes that facilitate the breakdown of the second messengers, cAMP and/or cGMP (Bender and Beavo, 2006). There are 11 distinct PDE families, with each typically consisting of several isoforms and/or splice variants. Molecules blocking the activity of this family of enzymes, collectively known as PDE inhibitors, have been a major focus of drug development, particularly for cardiovascular disease. Indeed, in the vasculature, PDE inhibitors exert several favourable effects including vasodilatation, inhibition of smooth muscle proliferation and prevention of platelet aggregation (Bender and Beavo, 2006).

Blockade of PDE5, which metabolizes cGMP exclusively, lowers systemic and pulmonary artery pressure under physiological conditions in animals and humans (Jackson *et al.*, 1999; Madhani *et al.*, 2006). Moreover, in animal models and patients with PH, PDE5 inhibitors cause larger reductions in pulmonary than systemic vascular resistance, thereby exhibiting relative selectivity for the pulmonary vasculature (Klinger *et al.*, 2006; Baliga *et al.*, 2008). In accord, PDE5 is found in abundance in the pulmonary vasculature and both expression and activity are elevated in PAH (Murray *et al.*, 2002; Sebkhi *et al.*, 2003). This favourable vasoactive profile of PDE5 inhibitors has culminated in the development and approval of sildenafil as a first-line therapy for PH; the drug elicits an improvement in several indices of disease severity

including pulmonary artery pressure, cardiac index, exercise capacity and WHO functional class (Galie *et al.*, 2005b). Sildenafil also appears to produce an overall beneficial effect on survival (Galie *et al.*, 2009b). Tadalafil, an analogous PDE5 inhibitor with a longer half-life, has also been recently licensed for the treatment of PAH (Galie *et al.*, 2009a). A third PDE5 inhibitor, vardenafil, is currently undergoing Phase III evaluation for the same indication.

Inhaled NO

The inhalation of exogenous NO gas decreases PAP and improves oxygenation in diverse forms of PAH, and is particularly effective in neonates suffering from persistent pulmonary hypertension (PPHN; Roberts *et al.*, 1992; Macrae *et al.*, 2004; Creagh-Brown *et al.*, 2009). However, long-term therapy with inhaled NO is complicated by the instability of NO gas, concerns regarding the development of methaemoglobinaemia (as NO binds avidly to, and oxidizes, the haem moiety) and marked rebound pulmonary hypertension following cessation of therapy (Ichinose *et al.*, 2004).

Novel therapeutic strategies

cGMP signalling

PDE5 inhibitors are an undoubted therapeutic advance, but their effects on PAP are small (approximately 5 mm Hg reduction). A significant cohort of PH patients does not respond to sildenafil treatment and in many individuals indices of disease severity do not differ from placebo approximately 12 months after initiation of therapy. Moreover, in patients who respond well to sildenafil, there is often a dose-dependent systemic hypotension that limits the beneficial effects of the drug. There is no evidence to suggest that the newer PDE5 inhibitors have a substantially greater effect than sildenafil, or that sildenafil resistant patients respond to other PDE5 inhibitors. Thus, there remains considerable opportunity to optimize interventions targeting cGMP-dependent signalling to improve the treatment of PH.

In PH, pulmonary vascular cGMP levels are decreased, either through impairment of NO bioavailability, guanylyl cyclase inactivation [enzymes that generate cGMP in response to NO and natriuretic peptides (Hobbs, 1997; Ahluwalia *et al.*, 2004; Potter *et al.*, 2006) or enhanced cGMP degradation by PDEs (Crawley *et al.*, 1992; Zhao *et al.*, 1992; Steudel *et al.*, 1997; Archer *et al.*, 1998). Accordingly, therapeutics targeted at augmenting cGMP levels have been shown to have therapeutic value in PH, either in animal models or patients with the disease.

NO donors. Attempts have been made to bypass the short half-life and indiscriminate chemical reactivity of (inhaled) NO, by developing more stable NO donors (e.g. NONOates), which spontaneously release defined amounts of NO when exposed to physiological pH. Daily nebulization with NONOates (e.g. diethylenetriamineNONOate; DEA-NO) has shown to be effective in animal models of PH (Vanderford *et al.*, 1994; Hampl *et al.*, 1996). Similarly, older NO donors such as glyceryl trinitrate administered by inhalation have been shown to be effective in reducing PAP in small clinical samples (Goyal *et al.*, 2006). Thus, delivery of NO via more sophisticated donor drugs may still prove to be efficacious in PH patients. Nonetheless, concerns regarding the lack of pulmonary selectivity, cGMP-independent cytotoxic effects and rebound pulmonary hypertension remain relevant.

Endothelial NO synthase augmentation. A further mechanism that may be exploited to treat PH is to improve endogenous NO bioavailability by augmenting the activity of endothelial NO synthase (eNOS). Expression and activity of eNOS, and the availability of a key redox co-factor, tetrahydrobiopterin (BH_4), are largely reduced in PH (Giaid and Saleh, 1995; Shaul *et al.*, 1997; Le Cras *et al.*, 1998; Khoo *et al.*, 2005), and mice with gene deletions in these systems are predisposed to the disease (Fagan *et al.*, 1999; Nandi *et al.*, 2005; Leiper *et al.*, 2007). However, under some circumstances, eNOS may be hyperactive in the pulmonary circulation in PH and, as a result of inadequate supply of BH_4, the enzyme uncouples to form superoxide rather than NO (Zhao *et al.*, 2009); this has the doubly detrimental effect of scavenging NO and producing direct cytotoxicity.

Several approaches focusing on eNOS/BH_4 have been evaluated for efficacy in PH. First, supplementation with BH_4 itself, or more promisingly an orally active, more stable form (6R-BH_4), is effective in augmenting endogenous BH_4 levels, restoring eNOS expression and reversing systemic hypertension (Landmesser *et al.*, 2003); similar effects may be achievable in PH. Second, the 'eNOS coupling agent', cicletanine, has shown modest beneficial effects in animal models of PH and humans with the disease (Jin *et al.*, 1992; Saadjian *et al.*, 1998), presumably by coordinating eNOS activity with BH_4 supply/binding and favouring the generation of NO over superoxide (although increasing the endogenous formation of PGI_2 and natriuretic peptides may also underlie these positive effects). Cicletanine is currently under phase II evaluation in patients with PAH. Thirdly, eNOS transcription enhancers may prove advantageous in PH, as they have shown in animal models to reverse the vascular remodelling and cardiac hypertrophy associated with left-sided heart failure (Westermann *et al.*, 2009), ischaemia-reperfusion injury [i.e. myocardial infarction (Sasaki *et al.*, 2006; Frantz *et al.*, 2009)], and atherosclerosis (Wohlfart *et al.*, 2008). Finally, the Pulmonary Hypertension and Cell Therapy trial, currently recruiting, is designed to test the safety and tolerability of autologous progenitor cell-based gene delivery of human eNOS in patients with severe PAH. This study may pave the way for more cell-based therapies for PAH, particularly because endothelial progenitor cells are thought to play a role in the pathogenesis of the disease (Toshner *et al.*, 2009), are a predictive biomarker and a novel therapeutic target (Yip *et al.*, 2008; Sun *et al.*, 2009; Toshner *et al.*, 2009; Fadini *et al.*, 2010).

Soluble GC activators. In order to harness the beneficial, cytoprotective effects of cGMP while circumventing the potentially detrimental cGMP-independent effects of NO, the development of directly acting sGC 'agonists' has progressed in rapid fashion. Soluble GC appears a good target in PH as the expression and activity of the enzyme is up-regulated in order to compensate for decreased NO bioavailability (Black *et al.*, 2001; Schermuly *et al.*, 2008; de

Frutos *et al.*, 2009) and genetic deletion of the enzyme results in an exaggerated response to hypoxia-induced PH (Vermeersch *et al.*, 2007).

Two different classes of sGC 'agonist' have been developed. First, sGC 'stimulators' or 'haem-dependent activators' (e.g. BAY 41-2272, BAY 41-8543, BAY 63-2521, riociguat) which stimulate the native Fe^{2+}-sGC and synergize with NO (Stasch *et al.*, 2002a,b). Second, sGC 'activators' or 'haem-independent activators' (e.g. BAY 58-2667, cinaciguat; HMR-1766, ataciguat) which activate the proposed Fe^{3+} or haem-free form of the enzyme and are additive with NO (Belik, 2009; Schmidt *et al.*, 2009; Stasch & Hobbs, 2009).

Both classes of drugs have been shown to have favourable effects on experimental PH (Dumitrascu *et al.*, 2006; Chester *et al.*, 2009; Weissmann *et al.*, 2009). Riociguat, an orally active sGC 'stimulator' is currently in Phase III trials for determination of clinical effectiveness in idiopathic PAH and CTEPH (Ghofrani *et al.*, 2010). However, a limitation of this sGC-centric strategy may be its lack of pulmonary selectivity, as shown by the systemic hypotension observed in earlier trials (Grimminger *et al.*, 2009). This is perhaps not unexpected. Soluble GC 'stimulators' synergize with NO and will therefore augment NO-dependent dilatation in all vascular beds. Moreover, in PH the bioavailability of NO in the pulmonary vasculature is known to be impaired, entailing that this synergy will predominate in the systemic, rather than pulmonary circulation. Nonetheless, these agents have exhibited a favourable profile in Phase II trials and offer a novel approach to treat PH; this therapeutic value may increase with inhalation or combination therapy to target the sGC 'stimulators' to the pulmonary circulation (Evgenov *et al.*, 2007). In addition, Phase III evaluation of sGC 'activators' (e.g. cinaciguat) that preferentially trigger the oxidized form of the enzyme, thought to be more prominent in diseased vasculature, may provide a more pulmonary-centred therapeutic approach in PH. Indeed, cinaciguat has already exhibited a favourable profile in patients with left-sided heart failure (Lapp *et al.*, 2009).

Natriuretic peptides. Atrial natriuretic peptide and brain natriuretic peptide are synthesized by and released from cardiac atrial and ventricular tissue, respectively, in response to stretch and elicit falls in blood volume and blood pressure (Ahluwalia *et al.*, 2004; Potter *et al.*, 2006). A third member of the family, C-type natriuretic peptide, is released from the vascular endothelium and regulates local blood flow in a paracrine fashion (Ahluwalia and Hobbs, 2005). Each natriuretic peptide acts on specific cell-surface natriuretic peptide receptors (NPR) in the vasculature which possess guanylate cyclase functionality. The increase in tissue cGMP in response to NPR activation brings about several cytoprotective effects including natriuresis, vasodilatation, and anti-hypertrophic and anti-proliferative activity [particularly in the heart (Oliver *et al.*, 1997)].

Genetic deletion of NPRs is associated with PH (Klinger *et al.*, 1999; Zhao *et al.*, 1999; Kuhn, 2004), while administration of exogenous natriuretic peptides has been shown to reduce hypoxia-induced PH (Klinger *et al.*, 1999); such observations provide the rationale for therapeutic modulation of natriuretic peptide signalling in PH. However, the short plasma half-life and negligible oral bioavailability make natriuretic peptides poor candidates for drug therapy. An alternative strategy is to increase endogenous natriuretic peptide levels by inhibiting the enzyme neutral endopeptidase (NEP), a major degradative pathway for natriuretic peptides (and other bioactive peptides) in the circulation (Okolicany *et al.*, 1992). This strategy has been proven to be effective in animal models both as monotherapy (Klinger *et al.*, 1993) and using the NEP inhibitor racecadotril in combination with a PDE5 inhibitor (Baliga *et al.*, 2008). Indeed, our data, both *in vitro* and *in vivo*, suggest that PDE5 is pivotal in terminating the cyclic GMP-dependent signalling in response to natriuretic peptides in the pulmonary vasculature, whereas other PDE isozymes regulate the vasorelaxant activity of natriuretic peptides in the systemic circulation (Baliga *et al.*, 2008). Therefore, by inhibiting PDE5 in PH, in which circulating natriuretic peptide concentrations are raised, it is possible to target the pulmonary vasculature and reduce pulmonary pressure. These observations explain the mechanism underpinning the pulmonary selectivity of PDE5 inhibitors and suggest that in PH, the release of natriuretic peptides represents a cytoprotective mechanism that reduces disease progression. This thesis is in accord with studies reporting increased expression and activity of PDE5 in the pulmonary circulation of patients with PH (Wharton *et al.*, 2005), that the beneficial effects of PDE5 inhibitors in models of PH are blunted in NPR-A knockout mice (Zhao *et al.*, 2003) and that, in patients with PH and animal models of the disease, acute infusion of natriuretic peptides in the presence of sildenafil synergistically reduces pulmonary artery pressure (Preston *et al.*, 2004; Klinger *et al.*, 2006). Thus, the therapeutic potential of manipulating natriuretic peptide bioactivity to reverse the haemodynamic abnormalities associated with PH holds great promise. This is true not only for the haemodynamic dysfunction, but also for attenuating the pulmonary vascular re-modelling that also characterises the disease. Natriuretic peptides inhibit pulmonary vascular smooth muscle proliferation and TGFβ-induced extracellular matrix expression *in vitro*, and prevent structural changes *in vivo* in animal models of PH (Jin *et al.*, 1990; Klinger *et al.*, 1998; 1999; Chen *et al.*, 2006; Li *et al.*, 2007).

The strategy of targeting neutral endopeptidase for the treatment of PH may also have the added benefit of slowing the breakdown of other protective peptides that will contribute to efficacy, including adrenomedullin and vasoactive intestinal peptide; both have been shown to be up-regulated in PH and to reverse disease progression in animal models (Shimokubo *et al.*, 1995; Gunaydin *et al.*, 2002; Matsui *et al.*, 2004; Qi *et al.*, 2007; Said *et al.*, 2007). However, NEP is also important in the metabolism of ET-1, which may offset some of its beneficial activity.

Other PDE inhibitors. PDE5 has received considerable attention in the context of PH due to the success of sildenafil and other selective inhibitors. However, other isozymes (e.g. PDE1 and PDE3) are also up-regulated in PAH, and might be suitable targets for therapy.

PDE 1 and PDE 3 (and splice-variants thereof) have been implicated in pulmonary vascular homeostasis and PH (Bender and Beavo, 2006). These enzymes hydrolyse cGMP and cAMP, although the PDE1A/1B splice variants have a higher affinity for cGMP (Bender and Beavo, 2006). PDE1A

and PDE1C expression and activity are up-regulated in animal models of PH and in tissues from patients with the disease (Evgenov *et al.*, 2006; Murray *et al.*, 2007; Schermuly *et al.*, 2007). Moreover, the selective PDE1 inhibitor, 8-methoxymethyl-isobutyl-1-methyl xanthine, reduces proliferation of human vascular smooth muscle cells (Rybalkin *et al.*, 2002) and reverses the haemodynamic and morphological aberrations associated with monocrotaline and hypoxia-induced PH (Schermuly *et al.*, 2007).

PDE 3A/3B expression and activity are also enhanced in PH (Murray *et al.*, 2002), and the presence of this 'cGMP-inhibited' PDE might underlie the synergistic cytoprotective activity of NO and prostacyclin in PH, and explain the benefit of co-administration of therapies promoting these pathways concomitantly [i.e. sildenafil and iloprost (Wilkens *et al.*, 2001)]. Indeed, a dual PDE3/4 inhibitor reverses monocrotaline-induced PH and synergizes with iloprost (Schermuly *et al.*, 2004; Dony *et al.*, 2008). The PDE3 inhibitor milrinone is currently being investigated for safety and efficacy in treatment of PPHN, but despite this potential, the increased mortality associated with the use of PDE3 inhibitors in (left) heart failure (Amsallem *et al.*, 2005) has limited the therapeutic enthusiasm for this approach in PH.

Anti-proliferative pathways

PAH is characterised by a shift in the proliferative/apoptotic balance and enhanced glycolytic metabolism (Mandegar *et al.*, 2004). Several growth factors, including platelet derived growth factor (PDGF), fibroblast growth factor 2, epidermal growth factor, vascular endothelial growth factor (VEGF) and, more recently, the non-canonical Wnt pathway have been implicated in the abnormal proliferation in PH (Oka *et al.*, 2007b; Hassoun, 2009; Izikki *et al.*, 2009). Levels of PDGF and its tyrosine kinase receptor PDGFR, are elevated in PAH patient lung samples (Perros *et al.*, 2008) and HIV-associated PH samples (Humbert *et al.*, 1998). VEGF levels are also increased in plexiform lesions in PAH patients (Cool *et al.*, 1999). These growth factors act as potent mitogens and chemoattractants, and through their transmembrane tyrosine kinase receptor pathways activate major proliferative signalling pathways such as the *ras*-mitogen activated protein kinase (MAPK) cascade, resulting in proliferation, migration and resistance to apoptosis (Hassoun, 2009). Consequently, this has led to increased interest in translation of anti-proliferative strategies, often originally developed for cancer therapy, to PAH patients.

Tyrosine kinase inhibitors

Imatinib (Gleevac) was initially developed as an anti-cancer therapy, predominantly chronic myelogenous leukemia, via inhibition of the oncogenic tyrosine kinase *Bcr-Alb*, but was later found to block the PDGFR and improve experimental PH (Schermuly *et al.*, 2005; Klein *et al.*, 2008). Several case studies of end-stage PH patients also suggest that treatment with imatinib can improve clinical conditions (Ghofrani *et al.*, 2005; Patterson *et al.*, 2006; Souza *et al.*, 2006; Tapper *et al.*, 2009; Ten *et al.*, 2009; Chhina *et al.*, 2010). This has led to a Phase III randomized, placebo-controlled clinical trial of imatinib in PAH (IMPRES), from which results are eagerly awaited. Nonetheless, there is some concern that long-term of imatinib could be associated with left ventricular dysfunction and heart failure (Kerkela *et al.*, 2006). Accordingly, other tyrosine kinase inhibitors have been developed and evaluated. Two such molecules are sunitinib and sorafenib, multi-kinase inhibitors, blocking PDGF, VEGF and other pro-proliferative signalling pathways. These molecules are currently being evaluated for safety and tolerability in Phase I, and are undoubtedly efficacious in animal models of PH (Klein *et al.*, 2008; Gomberg-Maitland *et al.*, 2010). However, it remains to be seen if such molecules are also associated with cardiotoxicity.

Several further molecules, often originally developed as anti-cancer agents, have also been investigated in animal models of PH, with positive outcomes, and are likely to lead to clinical evaluation in patients with the disease, particularly those molecules that are already licensed medicines. These include cell cycle inhibitors [e.g. rapamycin (Paddenberg *et al.*, 2007)], anti-apoptotic drugs [e.g. survivin inhibitors (McMurtry *et al.*, 2005)] and elastase inhibitors (Merklinger *et al.*, 2005).

Rho kinase inhibitors

The Rho kinase pathway participates in vasoconstriction elicited by numerous agents involved in PAH, including 5-HT, ET-1 and TXA_2 (Oka *et al.*, 2008). Rho is a small monomeric GTPase which activates Rho-associated kinase (ROCK) which in turn phosphorylates and inhibits myosin light chain phosphatase, which leads to prolonged, refractory vasoconstriction. Rho and ROCK also mediate smooth muscle cell proliferation, in a 5-HT-BMPR dependent pathway, and have been found to be elevated in smooth muscle cells from PAH patients (Do e Z *et al.*, 2009). Rho-kinase inhibitors have been shown to reduce PH in many animal models, including the monocrotaline rat, fawn hooded rats and chronic hypoxia/SUGEN exposure (Oka *et al.*, 2007a; Mouchaers *et al.*, 2010). In humans, Rho-kinase inhibition with fasudil shows modest, immediate reductions in PVR, but this inhibitor of Rho-kinase has to be administered by nebulization, that is, directly into the lungs, to avoid systemic hypotension (Ishikura *et al.*, 2006; Fujita *et al.*, 2010).

Bone morphogenetic protein signalling pathway

The discovery of the association between mutations in BMPR2 and PAH has led to increased interest in the BMP signalling pathway as a therapeutic target (Lane *et al.*, 2000). BMPR2 is a constitutively active serine-threonine kinase and a member of the TGFβ superfamily. In response to ligand, BMPR2 heterodimerises with one of four BMPR1 receptors (BMPR1A, BMPR1B, Alk1, Alk2), and phosphorylates the internal domain, triggering the cytosolic Smad protein signalling cascade (Yang *et al.*, 2005). Activation of the MAPK system [i.e. p38, extracellular signal regulated kinase 1/2 (ERK 1 /2) or Jun – N-terminal kinase] may also be an underlying mechanism. While BMPR2 mutations are relatively rare in non-familial PAH, dysfunctional BMPR signalling is often seen in PAH. For example, the expression of BMPR2 protein is markedly reduced in the lungs of patients with idiopathic

PAH with no detectable mutation in BMPR2 (Atkinson *et al.*, 2002), and cells isolated from PAH patients show altered response to BMP signalling (Morrell *et al.*, 2001). Reduced expression of BMPR2 is also found in the lungs of rats with monocrotaline-induced pulmonary hypertension (Morty *et al.*, 2007)

Results of BMPR2-targetted therapy in animal models have been mixed. Adenoviral gene delivery of BMPR2 failed to reverse monocrotaline-induced PH (McMurtry *et al.*, 2007), but intravascular administration of BMPR2 with an endothelial targeted vector in hypoxic rats produced better results (Reynolds *et al.*, 2007). In humans, BMPR2 mutations are thought to result in direct inactivation of the receptor or impaired trafficking of the receptor to the cell surface. Rescue strategies using viral vectors or chemical chaperones to overcome these aberrations are currently being investigated (Sobolewski *et al.*, 2008). Moreover, as pulmonary artery smooth muscle cells from familial PAH patients demonstrate increased sensitivity to TGFβ signalling, molecules aimed at blocking this pro-proliferative transduction system may be of therapeutic utility (Morrell *et al.*, 2001).

Peroxisome proliferator activated receptors

Recent observations suggest peroxisome proliferator activated receptors (PPARs) as another potential therapeutic target in PH. PPARγ is a downstream target of BMPR signalling and mediates the inhibitory effect of BMP on PGDF-induced smooth muscle cell proliferation (Hansmann and Zamanian, 2009) and PPARγ null mice develop PAH (Guignabert *et al.*, 2009). Moreover, PPARγ agonists have direct anti-inflammatory, anti-proliferative and pro-apoptotic effects (Hansmann *et al.*, 2007; 2008). Rosiglitazone, a PPARγ agonist, is effective in reducing the PH produced in ApoE$^{-/-}$ mice and reduces right ventricular hypertrophy and vascular remodelling in hypoxia-induced PH (Crossno *et al.*, 2007; Nisbet *et al.*, 2007). PPARs may also underpin some of the beneficial effects of prostacyclin analogues in PH (Ali *et al.*, 2006; Falcetti *et al.*, 2010; Harrington *et al.*, 2010).

5-HT signalling blockers

The 5-HT (serotonin) is a potent pulmonary vasoconstrictor. It was first implicated in the pathogenesis of PAH after outbreaks of the disease in patients using the anorexigenic drugs, aminorex and dexfenfluramine, appetite suppressants that inhibit 5-HT uptake (Dempsie *et al.*, 2008). 5-HT is synthesized in pulmonary artery endothelial cells by the enzyme tryptophan hydroxylase 1 (TPH1) and then then acts at one of several 5-HT receptor subtypes (primarily 5-HT$_{1B}$, 5-HT$_{2A}$ and 5-HT$_{2B}$) and through the 5-HT transporter (SERT), to mediate constriction and proliferation of pulmonary artery smooth muscle cells and fibroblasts (Welsh *et al.*, 2004). This results in a thickening of the medial layer and a narrowing of the lumen of the pulmonary artery and contributes to the pulmonary vascular remodelling associated with PAH. Downstream signalling molecules which play a role in 5-HT-signalling include ROCK, p38 and ERK1/2. Plasma 5-HT levels are elevated in PAH, as are SERT, 5-HT$_{1B}$ receptor and TPH1 expression in pulmonary artery smooth muscle and endothelial cells from PAH patients (MacLean and Dempsie, 2009). In addition, endothelial cells from PAH patients generate more 5-HT and proliferate more in response to 5-HT than control cells (Eddahibi *et al.*, 2001). Experimentally, the inhibition of SERT prevents 5-HT-dependent proliferation in cells, and reduces hypoxic PH in rodent models (Guignabert *et al.*, 2005; Song *et al.*, 2005; Zhai *et al.*, 2009; Zhu *et al.*, 2009). There is also evidence to suggest that 5-HT may interact with BMPR2 to provide a 'second hit' risk factor for PAH (Long *et al.*, 2006; Willers *et al.*, 2006). A single nucleotide polymorphism in the SERT gene has been identified in PAH patients that appears to associate with higher SERT expression and higher mean PAP, though this link has not been corroborated in subsequent studies (Eddahibi *et al.*, 2003; Machado *et al.*, 2006; Willers *et al.*, 2006; Roberts *et al.*, 2009). At present, a number of drugs modifying 5-HT signalling are under clinical evaluation for the treatment of PH, including terguride (5-HT$_{2A}$ and 5-HT$_{2B}$ receptor antagonist), PRX-08066 (selective 5-HT$_{2B}$ receptor antagonist) and escilatorpram (selective 5-HT re-uptake inhibitor, SSRI).

Renin-angiotensin-aldosterone axis

The renin-angiotensin-aldosterone system (RAAS) is up-regulated in PAH (Cargill and Lipworth, 1995) and steps in the RAAS system cascade appear to be viable therapeutic targets in PH. Indeed, the ACE inhibitor captopril was evaluated almost 20 years ago in PAH patients with some success (Alpert *et al.*, 1992). However, the development of more selective PAH therapies (i.e. specific to the pulmonary vasculature) has diverted attention from the RAAS as a viable target. Recently, the discovery that angiotensin-converting enzyme 2 (ACE2), a member of the vasoprotective arm of the RAAS, is up-regulated in both experimental models of PH and human PAH has refocused attention on this system (Ferreira *et al.*, 2009). ACE2 plays a regulatory role in the lung and activation of endogenous ACE2 shifts the balance from the vasoconstrictor, proliferative path (ACE/Angiotensin II/AT$_1$ receptor) to the vasoprotective anti-mitogenic path (ACE2/Angiotensin1-7/Mas) of the RAAS. Over-expression of ACE2 (by lentiviral gene delivery) or an ACE2 activator, XNT, reverses experimental PH (Ferreira *et al.*, 2009; Shenoy *et al.*, 2010).

Statins

Statins offer a novel approach to the treatment of PAH. This class of drugs have long been known to suppress vascular inflammation and vascular smooth muscle cell proliferation through a variety of mechanisms. In addition to lowering cholesterol via inhibition of 3-hydroxyl-3-methyl glutaryl CoA reductase, statins have been shown to have anti-proliferative, anti-thrombotic, anti-inflammatory and anti-oxidant effects, some of which may be secondary to cholesterol lowering. Statins, in particular simvastatin, have been reported to attenuate the development of PH in a number of experimental animal models (Nishimura *et al.*, 2002; Girgis *et al.*, 2003). Very recently, results from a double-

blind, randomized, placebo-controlled study of the effects of simvastatin added to optimized conventional care produced a small and transient early reduction in right ventricular mass and NT-proBNP levels in patients with PAH, but this was not sustained over 12 months (Wilkins *et al.*, 2010).

Ion channels

The haemodynamic dysfunction in PH patients also stems from abnormalities in the activity of ion channels that physiologically regulate local blood flow in the pulmonary circulation. For example, down-regulation of voltage-gated potassium channels, principally $K_v1.5$, appears to be a common feature of animal models of PH and in humans with the disease (Yuan *et al.*, 1998; Pozeg *et al.*, 2003). Targeting this potassium channel in PH is attractive because the facilitation of K^+ flux through this pore causes hyperpolarization, vasodilatation, and is also thought to promote apoptosis. The expression of $K_v1.5$ is inversely related to pulmonary vessel size, suggesting that therapy would concentrate on the small pulmonary arteries and thereby exert the greatest effect on pulmonary vascular resistance (Pozeg *et al.*, 2003). More recently, interest has arisen in the transient receptor potential (TRP) channel family. Experimental evidence suggests that TRPC6 expression and activity is up-regulated in PH and this leads to excessive Ca^{2+} entry into (pulmonary) vascular smooth muscle cells and vasoconstriction (Yu *et al.*, 2004), in addition to PDGF-mediated proliferation (Schermuly *et al.*, 2005). Moreover, an SNP in the TRPC6 promoter appears to associate with PH (Yu *et al.*, 2009). Indeed, reversal of TRPC6 up-regulation may represent an added benefit of sildenafil therapy in PH (Lu *et al.*, 2010). Finally, K_{ATP} channel activators such as iptakalim may have therapeutic utility in PH by producing pulmonary vasodilatation and preventing hypoxia- and ET-1-mediated pulmonary vascular smooth muscle cell proliferation (Xie *et al.*, 2004; Zhu *et al.*, 2008).

Cardiac-targeted therapy: β-adrenoceptor blockade

The major cause of death in PAH patients remains right ventricular failure, and perhaps one of the most-overlooked approaches in the treatment of the disease is cardiac-targeted therapies. Such strategies may have little or no direct effects on the pulmonary vasculature but prevent or reverse right heart dysfunction; it is reasonable to predict that such a tactic might make a major contribution to survival (Voelkel *et al.*, 2006).

The antagonism of β-adrenoceptors is a commonly used strategy in patients with left-sided systolic heart failure, in which mortality is reduced by approximately 30%, but is not used clinically in right heart failure (i.e. PAH). The $\alpha_1/\beta_1/\beta_2$-adrenoceptor blocker carvedilol and the selective β_1-adrenoceptor blockers, bisoprolol and metoprolol, reduce mortality in patients with left-sided systolic heart failure with a reversal of maladaptive cardiac remodelling, improved cardiac function and prevention of arrhythmias (Bristow *et al.*, 1996; Fowler *et al.*, 2007; MacGregor *et al.*, 2009). β-Adrenoceptor tachyphylaxis has also been demonstrated in PAH and may contribute to maladaptive right ventricular remodelling and the development of arrhythmias (Velez-Roa *et al.*, 2004). Carvedilol and metoprolol have been shown to reverse right ventricular remodelling and improve right ventricular function in experimental PH (Bogaard *et al.*, 2010), and the β-blocker arotinolol decreases both PAP and right ventricular hypertrophy, without altering systemic blood pressure, in a rat model of monocrotaline-induced PAH (Ishikawa *et al.*, 2009). Use of β-blockers in PAH has possible detrimental effects on haemodynamics and exercise capacity. While no specific clinical trial has been conducted to evaluate the efficacy and safety of β-blockers in PAH, a small cohort of porto-pulmonary hypertension patients were found to experience significant functional improvement following cessation of β-blocker therapy (Provencher *et al.*, 2006), suggesting a detrimental rather than beneficial outcome. Nonetheless, further investigation of this class of anti-hypertensive medicines may bring forth promising results in PAH patients.

Combination therapies

Since PH has a complex, multi-factorial aetiology, and the fact that current treatments (and the vast majority of the emerging therapies described previously) only target one aspect of the disease, modern approaches have focused on combining existing and newer therapies to bring about a significant improvement in outcome. This is a logical approach (based on the need for a combinatorial approach to adequately control systemic hypertension) and many studies suggest additive, if not synergistic, effects of combination therapy in PH (Schermuly *et al.*, 2001; Baliga *et al.*, 2008). Indeed, in clinical practice, combination therapy has become the default position even though trial evidence to support this strategy is limited. Small scale clinical evaluation of combinations of prostanoids, ERAs and PDE5 inhibitors have been tried with some success (Ghofrani *et al.*, 2002; Stiebellehner *et al.*, 2003; Stocker *et al.*, 2003; Hoeper *et al.*, 2004; Humbert *et al.*, 2004), with additional studies currently recruiting [e.g. COMPASS-2 (sildenafil plus bosentan), STEP (iloprost plus bosentan)]; however, validation of these combination therapies will require further larger scale trials. Moreover, these dual approaches have, to date, been restricted to combinations of existing therapies which are largely centred on the haemodynamic dysfunction. Newer therapies, targeting cell proliferation rather than vasodilatation, will necessarily entail novel combinations (as future trials will be on a background of existing treatment).

Combination therapy, however, has important implications for the cost of treating PH patients, which at present is approximately £45 000 per annum in the UK (National Institute for Health and Clinical Excellence). The partnership between academia, the pharmaceutical industry and healthcare providers has been successful in developing treatments for PH, but these drug costs pose a real challenge to healthcare systems. Exploring the potential of drug combination in PH that include generic medicines, such as simvastatin and racecadotril, has real potential for affordable drug development.

Conclusions

Advances in the treatment of PH over the past decade have enabled physicians to substantially improve the prognosis, yet the mortality rate remains high. Existing treatments are based predominantly on vasodilatation, whereas many emerging therapies are aimed at cell proliferation and re-modelling (Figure 2). There is great optimism that this alternative strategy will yield superior results, either alone or in combination.

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Conflicts of interest

None.

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