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 dysfunction 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.

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Abbreviations
AC, adenylate cyclase; ACE, angiotensin converting enzyme; BH₄, 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; Kᵥ, 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; PGI₂, 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; SSR1, selective 5-HT reuptake inhibitor; TGFβ, transforming growth factor β; TRK, tyrosine kinase receptor; TRPC6, transient receptor potential C6; TXA₂, thromboxane A₂; 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 1970s, 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 pathological 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. Connective 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 histiocytosis, 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.
Emerging therapies for pulmonary hypertension

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 (PGI₂).

**Ca²⁺ channel blockers**

L-type Ca²⁺ channel blockers (CCB), such as nifedipine, diliazem 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**

PGI₂ and thromboxane A₂ (TXA₂) are arachidonic acid metabolites with opposing vasoactivity. In PAH, the balance is shifted towards vasoconstrictor, pro-proliferative TXA₂ from vasoconstrictor, anti-proliferative PGI₂ (Christman et al., 1992; Tuder et al., 1999). This relative impairment in PGI₂-dependent signalling in PAH leads to the development of analogues that would mimic the cytoprotective activity of this prostanoid and restore the balance between PGI₂ and TXA₂. The beneficial activity of prostacyclin (analogues) in PH is presumed to be via activation of the Gc-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, treprostinil 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; ETₐ receptors on vascular smooth muscle cells cause vasoconstriction and proliferation, while ETₐ 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 ETₐ/ETₐ, ambrisentan (ETₐ > ETₐ) and sitaxsentan (ET₁, > > ETₐ) 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 ET₁ receptor selective agents (selective ET₁ antagonists should possess a theoretical advantage in not preventing the production of NO via ET₁ receptor activation on endothelial cells) are still lacking. Macitentan, a novel ET₁/ETₐ receptor antagonist, is currently in a phase III trial in PAH (SERA PHIN), after producing a promising haemodynamic profile in a smaller Phase II trial in hypertensive patients (Raja, 2010).
**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 (PHHN; 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 methaemoglobinemia (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 (BH4), are largely reduced in PH (Giaid and Saleh, 1995; Shaul et al., 1997; Le Cras et al., 1998; Kho 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 BH4, 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/BH4 have been evaluated for efficacy in PH. First, supplementation with BH4 itself, or more promisingly an orally active, more stable form (6R-BH4), is effective in augmenting endogenous BH4 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), preferably by coordinating eNOS activity with BH4 supply/binding and favouring the generation of NO over superoxide (although increasing the endogenous formation of PGI2 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 PH, 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 (Vermeesch 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⁺⁺-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⁺⁺ 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 elicited falls in blood volume and blood pressure (Ahlulwalia 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 (Ahlulwalia 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, vaso dilatation, and anti-hypertrophic and anti-proliferative activity [particularly in the heart (Olive 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 (Okolica 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 raccadotril 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; Matsu 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 (Wilkins 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 receptor (EGF) and, more recently, the non-canonical Wnt pathway have been implicated in the abnormal proliferation in PH (Oka et al., 2007b; Hassoun, 2009; Iizki 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 chemotactants, 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 (Gleevec) was initially developed as an anti-cancer therapy, predominantly chronic myelogenous leukaemia, 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; Chihina 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 (McMurty 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 TXA₂ (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 phosphorylation, 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 BMP2 and PAH has led to increased interest in the BMP signalling pathway as a therapeutic target (Lane et al., 2000). BMP2 is a constitutively active serine-threonine kinase and a member of the TGFβ superfamily. In response to ligand, BMP2 heterodimersises 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 BMP2 mutations are relatively rare in non-familial PAH, dysfunctional BMPR signalling is often seen in PAH. For example, the expression of BMP2 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 TGF β 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 anorexigens 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-HT1A, 5-HT2A and 5-HT2B) 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-HT1A 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-HT2A and 5-HT2B receptor antagonist), PRX-08066 (selective 5-HT1B receptor antagonist) and escloprorpram (selective 5-HT re-uptake inhibitor, SSRl).

**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-convertimg 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/AT1 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,1.5, 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,1.5 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 (TRPC) channel family. Experimental evidence suggests that TRPC6 expression and activity is up-regulated in PH and this leads to excessive Ca2+ 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,12 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 α1/β/β2-adrenoceptor blocker carvedilol and the selective β1-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 atorvastol decreases both PAP and right ventricular hypertrophy, without altering systemic blood pressure, in a rat model of monocrotaline-induced PAH (Isikawa 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 has 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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