



## REVIEW

# Pathways in pulmonary arterial hypertension: the future is here

Olivier Sitbon\* and Nicholas W. Morrell#

**ABSTRACT:** It is well established that the endothelin, nitric oxide and prostacyclin pathways play an important role in the development of pulmonary arterial hypertension (PAH). Indeed, the therapeutic options currently available for the management of PAH all act on one of these mechanistic pathways.

However, this is an exciting time for both clinicians and scientists, as increased understanding of the mechanisms involved in the pathogenesis and progression of PAH has resulted in the development of a number of novel therapeutic options.

This article highlights how the introduction of new compounds such as macitentan, riociguat and selexipag, which act on the endothelin, nitric oxide and prostacyclin pathways, respectively, have the potential to further improve the prognosis for patients with PAH.

**KEYWORDS:** Endothelin, nitric oxide, prostacyclin, pulmonary arterial hypertension, survival

**P**ulmonary arterial hypertension (PAH) is a disease often considered to be driven by vasoconstriction. However, one cannot overlook the associated, profound remodelling of the vascular wall that mainly contributes to the pathogenesis of PAH. New medicines should seek to ameliorate the intimal fibrosis, endothelial cell proliferation, medial thickening and smooth muscle cell hypertrophy and hyperplasia seen in the pulmonary vasculature of patients with PAH [1]. Major therapeutic advances have been made in the past 20 yrs, with the introduction of novel compounds that target the three key pathways involved in the development and progression of PAH, namely: the endothelin (ET), nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>) pathways (fig. 1) [2]. Research is ongoing, and a new wave of medicines, with more potent antihypertensive and antiproliferative effects, are expected to be launched in the near future.

### THE ET PATHWAY

It is well established that ET-1 is a key mediator of PAH, driving pathological changes in the lung that lead to pulmonary vascular remodelling [3–5]. The effects of ET-1 are mediated *via* two ET receptor subtypes, ETA and ETB, to which ET-1 binds with high affinity [6, 7]. ETA receptors are found in smooth muscle cells while ETB receptors are located in both endothelial cells and smooth muscle cells. ET-1, released from the endothelium,

acts primarily on the underlying smooth muscle cells to cause vasoconstriction and proliferation. In addition, ET-1 acts on fibroblasts to induce contraction, proliferation and fibrosis, and on the endothelium itself to cause proliferation, vasodilation (*via* NO and PGI<sub>2</sub>) and vasoconstriction (*via* thromboxane A<sub>2</sub>). All three of these ET-driven processes play an important role in lung vascular and structural remodelling (fig. 2) [8].

A greater understanding of the ET system has elucidated a role for autocrine/paracrine signalling in the ET system and PAH pathway. The evidence to support this hypothesis is reinforced by a number of observations. For instance, cleavage of big ET-1 to form mature ET-1 is known to occur *in situ* [7, 9, 10], and circulating plasma levels of ET-1 are below the pharmacological threshold levels required to elicit vasoconstriction. This is suggestive of a local site of action [6]. Additional support for the importance of autocrine/paracrine signalling includes the polarised secretion of ET-1, whereby 80% of the ET-1 produced by the endothelial cells is released basolaterally towards the vessel wall, and hence the tissue, while only 20% is released into the bloodstream [11]. Furthermore, DAVIE *et al.* [12] reported an increased number and density of ET receptors in the small pulmonary arteries of patients with idiopathic PAH and PAH associated with congenital heart disease. Not only does there seem to be a high concentration of ET receptors, there also seems to

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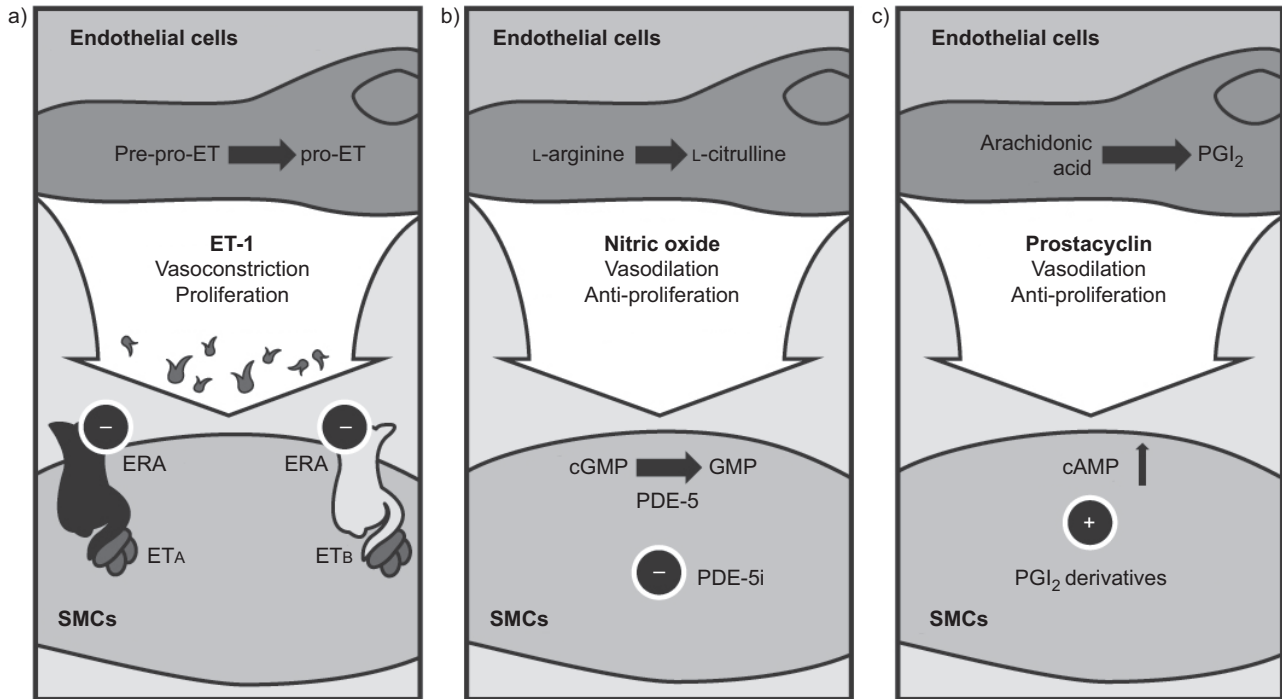
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### PROVENANCE

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**FIGURE 1.** Key pathways involved in the pathogenesis of pulmonary arterial hypertension: a) endothelin (ET) pathway; b) nitric oxide pathway; and c) prostacyclin (PGI<sub>2</sub>) pathway. ERA: ET receptor antagonist; ET<sub>A</sub>/ET<sub>B</sub>: ET receptor subtypes A and B; SMCs: smooth muscle cells; cGMP: cyclic guanosine monophosphate (GMP); PDE-5: phosphodiesterase type-5; PDE-5i: PDE-5 inhibitor; cAMP: cyclic adenosine monophosphate. Reproduced from [2] with permission from the publisher.

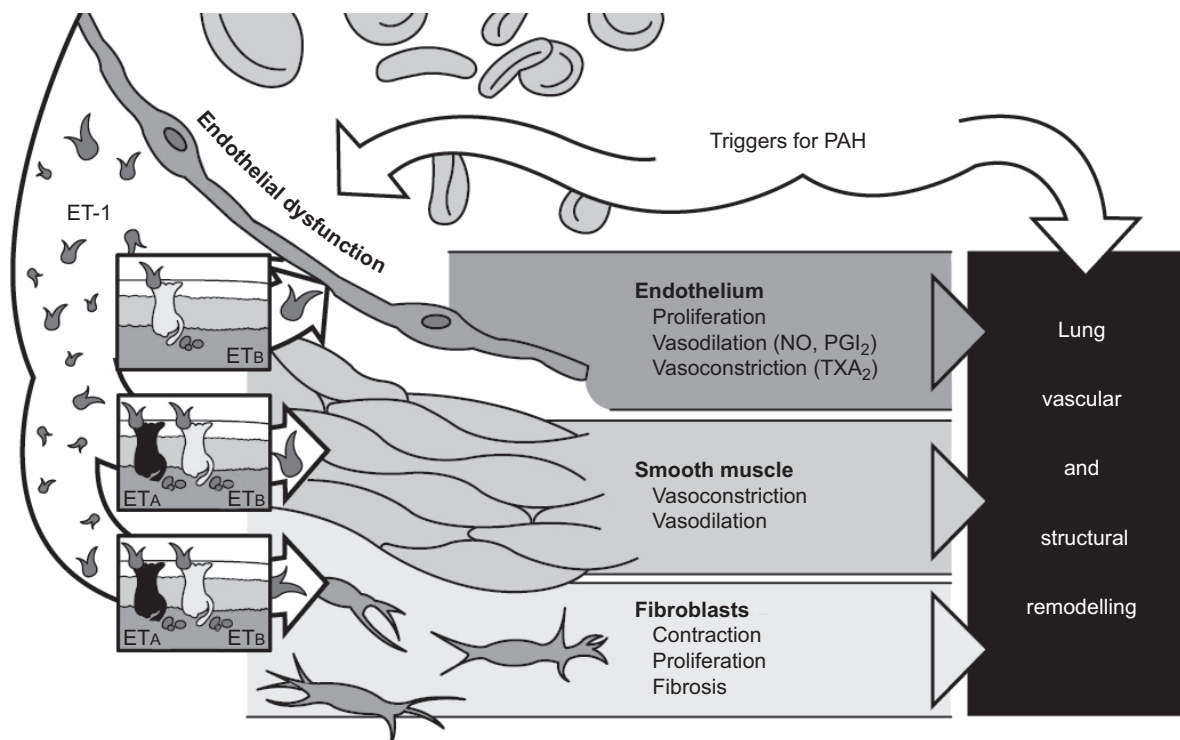
be continuous occupancy of these receptors by their agonist [13, 14]. These findings have prompted the development of new endothelin receptor antagonists (ERAs), such as macitentan, which demonstrate enhanced tissue penetration, improved receptor binding/affinity and superior efficacy compared with the currently available drugs (bosentan and ambrisentan).

Macitentan is a novel dual ERA currently in phase III of clinical development. As mentioned previously, the majority of ET-induced effects occur deep within the tissues so it is important that any new, potential antagonist of ET can readily penetrate the lipid bilayer. Results from *in vitro* studies demonstrated that macitentan has a higher ionisation dissociation constant (pK<sub>a</sub>=6.2) than bosentan (pK<sub>a</sub>=5.1) or ambrisentan (pK<sub>a</sub>=3.5) [15]. At a pK<sub>a</sub> of 6.2, 6% of macitentan is non-ionised at physiological pH, which, in turn, facilitates passage across the lipid bilayer. This means that macitentan has a greater affinity for the lipid phase compared with other ERAs and, accordingly, has greater tissue-targeting potential. This supposition is supported by the results from an experimental rat model of pulmonary fibrosis. In this study, micro-autoradiographs indicating the localisation of each drug demonstrated greater tissue penetration with macitentan compared with bosentan [16].

Studies in animal models suggest that macitentan has improved efficacy compared with other ERAs. Macitentan was shown to be more efficacious than bosentan in reducing the development of pulmonary fibrosis and right ventricular hypertrophy in the rat model of pulmonary fibrosis associated with PAH [16]. In addition, a study using the deoxycorticosterone acetone-salt rat model of hypertension investigated the effect of acute oral macitentan *versus* bosentan administration on mean arterial

pressure. Macitentan was shown to be 10-fold more potent than bosentan and displayed a two-fold longer duration of action [15, 17]. Pre-clinical data also suggest beneficial long-term effects of macitentan; macitentan (30 mg·kg<sup>-1</sup>·day<sup>-1</sup>) elicited a significant improvement in survival in a monocrotaline (MCT) rat model of PAH when compared with untreated controls (fig. 3) [15]. Survival at 42 days in rats was 83% with macitentan and 50% with the vehicle control (p=0.002), which represents a 66% reduction in mortality.

Safety and tolerability with ERAs is an important consideration, especially as it is not known whether patients treated with these agents may experience an increased incidence of hepatic side-effects [8]. There are several known mechanisms for this class effect, including inhibition of the bile salt export pump (BSEP). The BSEP is the major transport protein for bile salt export and its inhibition can lead to elevated liver enzymes. Bosentan inhibits BSEP, resulting in disruption of bile salt homeostasis, which can cause hepatic cholestasis and liver damage [18]. Unlike bosentan, *in vivo* data demonstrate that neither macitentan nor its active metabolite inhibit BSEP to a clinically relevant extent [17]. In contrast to bosentan, macitentan is given at a significantly lower dose (10 mg *versus* 125 mg *b.i.d.*) and is mainly excreted into urine. Furthermore, in the Macitentan Use in an Idiopathic Pulmonary Fibrosis Clinical (MUSIC) study (a phase II trial of macitentan in idiopathic pulmonary fibrosis), the incidence of liver enzyme elevations with macitentan was similar to placebo (3.4% and 5.1%, respectively) [19]. Based on these experiments, pivotal toxicology data and clinical trial results, it is expected that macitentan and its active metabolite are less likely to cause elevations in liver enzymes than bosentan. Pharmacokinetic



**FIGURE 2.** Endothelin (ET)-1 plays an important role in vascular remodelling. PAH: pulmonary arterial hypertension; ET<sub>A</sub>/ET<sub>B</sub>: ET receptor subtypes A and B; NO: nitric oxide; PGI<sub>2</sub>: prostacyclin; TXA<sub>2</sub>: thromboxane A<sub>2</sub>.

data from phase I studies support a once-daily dosing regimen for macitentan and demonstrate that it can be taken irrespective of food intake and without drug–drug interactions with warfarin, sildenafil, ketoconazole or cyclosporine [20, 21].

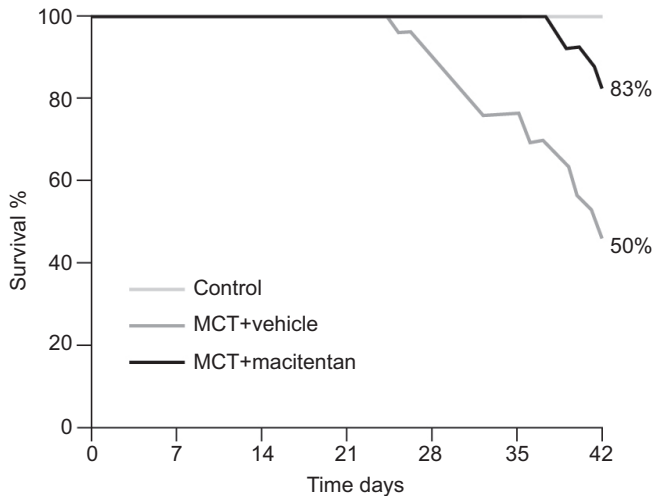
The results from the pre-clinical programme and from phase I and II studies suggest that macitentan is efficacious and well tolerated [22]. The recently completed phase III Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome (SERAPHIN) study (Clinicaltrials.gov NCT00660179) was undertaken in 39 countries and randomised 742 patients with symptomatic PAH. SERAPHIN was a double-blind, randomised, placebo-controlled, event-driven study in which patients were randomised 1:1:1 to receive either 3 mg or 10 mg macitentan or placebo once daily, in addition to their current (if any) PAH medication (although patients receiving other ERAs were excluded). Historically, PAH trials have been short term (typically 12–16 weeks) and the primary end-points have focused on symptomatic or functional end-points such as the 6-min walk distance (6MWD). In contrast, SERAPHIN was an event-driven study whose primary end-point is time to first morbidity or mortality event. This was the largest, prospective-controlled study in PAH to date and will provide much needed data on the long-term treatment of patients with PAH.

### THE NO PATHWAY

PAH is associated with impaired production of the endothelium-derived vasodilator, NO [23]. In healthy individuals, NO acts on smooth muscle cells to induce vasodilation and inhibit proliferation by increasing production of the secondary messenger cyclic

guanosine monophosphate (cGMP) *via* activation of soluble guanylate cyclase [24, 25]. Treatment of pulmonary hypertension (PH) with NO-releasing agents such as nitrates has failed to produce beneficial long-term effects as, in most cases, negligible pulmonary vasodilation was counterbalanced by significant peripheral reduction in vascular resistance and reflex tachycardia [26], both of which are poorly tolerated by patients with severe PH. An alternative therapeutic strategy targets downstream components of the NO signalling pathway by inhibiting phosphodiesterase type-5 (PDE-5), the enzyme that catalyses the conversion of cGMP to GMP [27]. Sildenafil and tadalafil have been the lead substances in this group of agents, showing both acute and long-term beneficial effects in patients with PAH [28–30]. However, PDE-5 inhibition is not effective in all patients with PH [31]; therefore, the full therapeutic potential of inducing the NO signalling pathway remains to be exploited.

Riociguat offers a new mode of action for the treatment of PAH by acting directly on soluble guanylate cyclase, stimulating the enzyme and increasing sensitivity to low levels of NO [32]. Phase II proof-of-concept studies in PAH and chronic thromboembolic PH (CTEPH) have demonstrated that riociguat was generally well tolerated and improved exercise capacity, symptoms and pulmonary haemodynamics in patients with PAH or CTEPH [32, 33]. Phase III randomised controlled trials are currently underway. The Pulmonary Arterial Hypertension sGC-Stimulator Trial (PATENT) is a randomised, placebo-controlled trial that investigates the efficacy and safety of riociguat in patients with PAH (Clinicaltrials.gov NCT00810693). Patients were treated with riociguat or placebo for 12 weeks. The primary end-point in PATENT is 6MWD at 16 weeks. PATENT-2 is the open-label



**FIGURE 3.** Effect of macitentan on survival in a monocrotaline (MCT) rat model of pulmonary hypertension.  $p=0.002$ . Reproduced from [15] with permission from the publisher.

extension study in which patients are invited to participate. The Chronic Thromboembolic Pulmonary Hypertension sGC-Stimulator Trial (CHEST) is a randomised, placebo-controlled trial looking at the efficacy and safety of riociguat *versus* placebo in CTEPH patients (Clinicaltrials.gov NCT00855465). Again, the primary end-point is the change in 6MWD. On completion of CHEST-1, patients are invited to enter the open-label extension trial, CHEST-2. The results of the riociguat phase III studies, CHEST-1 and PATENT-1, are expected in mid-to-late 2012.

### THE PGI<sub>2</sub> PATHWAY

The PGI<sub>2</sub> pathway has an established role in the pathogenesis of PAH [2, 34]. This pathway is activated when PGI<sub>2</sub> stimulates the IP receptor, leading to increased cyclic adenosine monophosphate and resulting in vasodilatory and antiproliferative effects [34]. PAH patients have reduced levels of endogenous PGI<sub>2</sub> and reduced expression of PGI<sub>2</sub> synthase in the lung [35, 36]. Several PAH-specific therapies have been developed that target the PGI<sub>2</sub> pathway [34]. However, there are significant limitations associated with currently approved therapies, including inconvenient modes of administration (by continuous intravenous or subcutaneous injection, or inhalation), short half-life and non-specific receptor interactions [37, 38]. Currently, there are no licensed oral PGI<sub>2</sub> or PGI<sub>2</sub> analogues available in Europe or the USA. Parenteral prostanoids are available but their uptake has been poor, presumably because of difficulties with administration. This was clearly illustrated in the Registry to Evaluate Early and Long-Term PAH disease (REVEAL) study, which demonstrated that ~40% of patients who died from PAH had not received prostanoid therapy at the time of death [39].

Clearly, an oral PGI<sub>2</sub> or PGI<sub>2</sub> analogue would be a welcome addition to the portfolio of drugs used in the management of patients with PAH. Several oral therapies that target the PGI<sub>2</sub> pathway are in clinical development. One of these, oral treprostinil, has been studied in three phase III, multinational, placebo-controlled trials; as monotherapy in FREEDOM-M and in combination with background therapy in FREEDOM-C and FREEDOM-C2 (Clinicaltrials.gov NCT00325403, NCT00325442

and NCT00887978, respectively) [40]. The primary end-point for all three trials was change in 6MWD at 12 weeks in FREEDOM-M and at 16 weeks for FREEDOM-M and FREEDOM-C/C2. In FREEDOM-M, treprostinil improved 6MWD in comparison with placebo (treatment effect of +23 m;  $p=0.0125$ ). Among the secondary end-points, 6MWD at week 8 and combined 6MWD and Borg dyspnoea score were significantly improved by treprostinil. However, treprostinil did not significantly affect World Health Organization (WHO) functional class or time to clinical worsening. In FREEDOM-C and FREEDOM-C2, preliminary analyses have shown that the primary end-points were not met [41, 42]. Another molecule that was under clinical investigation was beraprost. Beraprost is an orally active PGI<sub>2</sub> analogue developed for the treatment of PAH. However, it has a short half-life (~1 h) [43] and only modest, transient clinical benefits [44]. A recent phase II trial with a sustained-release formulation of beraprost failed to meet its primary end-point and, as a result, plans to commence a phase III trial with beraprost (sustained-release formulation) have been suspended (Clinicaltrials.gov NCT00989963) [45–47].

On a more optimistic note, there are promising clinical trial results with the first-in-class non-prostanoid IP receptor agonist selexipag [34, 38]. Selexipag is a potent, orally active molecule which is rapidly hydrolysed to an active metabolite, ACT-333679. Both selexipag and its metabolite are highly selective for the IP receptor compared with other prostanoid receptors such as EP, DP, FP and TP [2, 34–36, 38]. This selectivity for the IP receptor offers the potential for improved tolerability with selexipag, as side-effects (*e.g.* nausea and vomiting) that might result from activation of the other prostanoid receptors may be minimised [48]. In addition, the selexipag metabolite has a half-life of 7.9 h, thus permitting oral dosing twice daily [38].

Selexipag has been studied in a randomised, double-blind, placebo-controlled, phase II, proof-of-concept trial in 43 patients with PAH [49]. Approximately one-third of patients were receiving both an ERA and sildenafil as background therapy. Selexipag was up-titrated in 200 µg *b.i.d.* increments to the maximum tolerated dose. More than 60% of patients were on a final selexipag dose of 600–800 µg *b.i.d.* Throughout the study selexipag was well tolerated with the majority of adverse events being mild or moderate in severity. Selexipag significantly lowered pulmonary vascular resistance (PVR) in comparison with placebo at week 17 (treatment effect of -30.3%;  $p=0.0045$ ). In addition, there was an improvement in 6MWD (treatment effect of +24.7 m), although this did not reach statistical significance. Compared with placebo, selexipag treatment was associated with a significant increase in cardiac index (treatment effect of +0.5 L·min<sup>-1</sup>·m<sup>-2</sup>;  $p=0.01$ ). Further investigation of selexipag is ongoing in the double-blind, randomised, placebo-controlled, phase III GRIPHON (Prostaglandin (PGI<sub>2</sub>) Receptor Agonist in Pulmonary Arterial Hypertension) study. GRIPHON has a clinically relevant and highly robust primary end-point of time to first morbidity/mortality event and will provide vital information on the long-term effects of selexipag in patients with PAH (Clinicaltrials.gov NCT01106014).

### ADVANCES IN OTHER PATHWAYS

#### Tyrosine kinase inhibitors

Platelet-derived growth factor (PDGF) is a serum growth factor for several cell types, including vascular smooth muscle cells

and fibroblasts [50]. As a potent mitogen, PDGF exerts its effects *via* two receptor tyrosine kinases: PDGFR- $\alpha$  and PDGFR- $\beta$  [51]. Evidence suggesting a role for PDGF in the pathophysiology of PAH implies that inhibition of the PDGF pathway may represent a therapeutic target for the development of novel pharmacological strategies in PAH [52].

Imatinib is a tyrosine kinase inhibitor that was initially designed to treat chronic myelogenous leukaemia [53]. Imatinib can inhibit vascular smooth muscle cell proliferation and migration and, therefore, may suppress PDGF-induced vascular remodelling [50, 51]. In support of this, imatinib has been shown to reverse pulmonary arterial smooth muscle cell proliferation and neointima formation in rats and mice [52]. Moreover, in rats with MCT-induced PH, imatinib administered daily over 2 weeks resulted in 100% survival compared with only 50% in sham-treated rats. In humans, several case reports have shown promising results for imatinib as a treatment for PAH [54–56]. However, one must caution that some of these patients had severe PAH and had exhausted all of the existing, traditional treatment options [54, 55].

The effects of imatinib in PAH were investigated in a randomised, double-blind, placebo-controlled phase II study [57]. 59 patients who remained in WHO functional class II–IV following >3 months of PAH-specific therapy (including PGI<sub>2</sub> analogues, ERAs or PDE-5 inhibitors) received treatment with oral imatinib (target dose 400 mg·day<sup>-1</sup>) or placebo. After 24 weeks, the study failed to meet its primary efficacy end-point of improvement in 6MWD. However, some secondary end-points, including PVR, were improved. A *post hoc* analysis, which stratified patients according to baseline median PVR, demonstrated improvements in 6MWD and haemodynamics in those patients treated with imatinib who had a baseline PVR  $\geq 1,000$  dyn·s·cm<sup>-5</sup>. Although caution is warranted in interpreting the results of unplanned *post hoc* analyses, it is proposed that disease severity needs to reach a certain threshold for imatinib to be effective.

On the basis of the phase II data, the 24-week, double-blind, phase III Imatinib in Pulmonary Arterial Hypertension, a Randomized Efficacy Study (IMPRES) was initiated [58]. The aim of this study was to evaluate imatinib as an add-on therapy for the treatment of severe PAH. The IMPRES study included 202 patients with elevated PVR ( $\geq 800$  dyn·s·cm<sup>-5</sup>), despite treatment with at least two other PAH-specific medications. The results of the study demonstrated a significant improvement in 6MWD with imatinib *versus* placebo. Although significant improvements were shown for the secondary end-points of cardiac output, PVR and N-terminal pro-brain natriuretic peptide levels, this was not the case for the time to clinical worsening. There was also no significant change in WHO functional class, CAMPHOR (Cambridge Pulmonary Hypertension Outcome Review) quality of life score or Borg dyspnoea score. The overall incidence of adverse events was similar for imatinib and placebo. However, discontinuations due to serious adverse events were more frequent with imatinib. The serious adverse events included oedema, anaemia and subdural haematoma. The IMPRES extension study is currently underway to evaluate the long-term safety, tolerability and efficacy of imatinib in patients with severe PAH (Clinicaltrials.gov NCT01117987).

Tyrosine kinase inhibitors may represent a promising class of compounds for the future treatment of PAH. However, there are still a number of issues, such as the safety and tolerability profile, which need to be addressed. However, an important consideration is that currently imatinib cannot be used outside of clinical trials. In addition, not all tyrosine kinase inhibitors have the same intracellular target. It may be that molecules such as dasatinib, which may actually induce severe PH in patients with chronic myelogenous leukaemia, may target different receptor tyrosine kinases than a compound such as imatinib that appears to show benefit in PAH [59]. A clearer understanding of the tyrosine kinase pathway and receptors will be key to the future development of effective therapeutic agents in this class.

### Terguride

Terguride is an oral, potent antagonist of serotonin (5-HT<sub>2A</sub> and 5-HT<sub>2B</sub>) receptors. Serotonin is a signalling molecule with many functions in the body. In blood vessels it stimulates proliferation of smooth muscle cells and constriction of the vessel wall which can induce PAH. Terguride binds to the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors and inhibits the actions of serotonin on the vasculature, thereby attenuating the development of PAH. In 2008, terguride was granted orphan drug status for the treatment of PAH. Recent results from a double-blind, randomised, phase IIa study showed no overall significant effect of terguride compared with placebo on PVR or secondary end-points, and the rate of severe and serious adverse events was higher with terguride. However, improvements in PVR among subjects already on PAH therapy with ERAs warrant further investigation [60].

### SUMMARY AND CONCLUSIONS

This is an exciting time for clinicians and scientists involved in PAH research as several promising therapeutic options are in development for the treatment of this disease. Major advances have been made, with the introduction of novel compounds, such as macitentan, riociguat and selexipag, which target the three key pathways (ET, NO and PGI<sub>2</sub>, respectively) involved in the pathogenesis and progression of PH. A number of important clinical trials in PAH are underway, the results of which are eagerly anticipated. The landmark SERAPHIN study, with the novel dual ERA macitentan, will provide much-needed information on the long-term effects of therapy on mortality and morbidity outcomes for patients with PAH. The GRIPHON study, with the IP receptor agonist selexipag, will give insight into the sustained effect of oral PGI<sub>2</sub> agonists on long-term disease progression and it is hoped that PATENT-1 and CHEST-1 will elucidate the putative, therapeutic role of the soluble guanylate cyclase activator, riociguat, in patients with PH. Clearly, with so much effort and activity in this field, it is hoped that, in the not too distant future, new treatment(s) will be available that will improve the prognosis for patients with PAH.

### STATEMENT OF INTEREST

O. Sitbon has relationships with drug companies including Actelion, Bayer HealthCare, GSK, Eli Lilly, Pfizer and United Therapeutics. In addition to being an investigator in trials involving these companies, relationships include consultancy service and membership of scientific advisory boards. N.W. Morrell has received honoraria from Actelion for speaking at educational meetings.

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