Future perspectives in pulmonary arterial hypertension

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ABSTRACT While there have been advances in the field of pulmonary arterial hypertension (PAH), disease management remains suboptimal for many patients. The development of novel treatments and strategies can provide opportunities to target other mechanisms that play a role in the complex pathobiology of PAH outside of the three main pathophysiological pathways. In this review, we highlight some of the potential PAH therapies or techniques that are being, or have been, investigated in phase II clinical trials. This review also discusses potential points for consideration in the development of novel therapies that target putative disease mediators or modifiers.

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Introduction

Pulmonary arterial hypertension (PAH) has evolved from a disease with limited treatment options to one where numerous therapies that target three key pathophysiological pathways in the disease (*i.e.* the prostacyclin, endothelin and nitric oxide pathways) are available [1]. All three pathways can now be targeted with approved drugs that significantly improve long-term outcomes [2–4]. As a result of recent developments in the field of PAH treatment, these treatment options are becoming more accessible. However, despite the advances, disease management remains unsatisfactory for many PAH patients [5] and a large number are still only treated with one PAH-specific therapy. To provide the best outcomes for patients with PAH, there is a clear need to ensure that patients are diagnosed as quickly and accurately as possible, and are optimally managed using the treatments that are currently available. One way to optimise the management of PAH patients is the use of combination therapy, which is discussed in greater detail in the article by Sitbon and Gaine [6]. Another approach to the optimisation of PAH management is the development of novel treatments and therapeutic strategies that will provide further opportunities to improve outcomes for PAH patients.

The diverse and complex mechanisms underlying the pathogenesis of PAH offer the potential for new therapies that target pathways beyond the three well-established pathways. However, only 7–8% of

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cardiopulmonary drugs reach the market [7], with >50% of failures occurring in early clinical development due to a lack of efficacy [8, 9]. Therefore, it is important that novel targets in PAH are examined carefully, using well-designed trials to enable the most meaningful data to be obtained. In this review, we will explore potential novel targets for the management of PAH, focusing on immune and inflammation pathways, mitochondrial dysfunction, bone morphogenetic protein receptor type 2 (BMPR2) signalling, iron-deficient states and pulmonary artery denervation (PADN). In particular, therapies that have progressed to, or are already being investigated in, phase II clinical trials will be highlighted. We will also discuss points to consider in the development of novel therapies for PAH, including lessons that can be learned from previous clinical trials.

Potential PAH therapies currently in development

Therapies targeting inflammation and immunity

A number of observations in PAH patients have implicated a dysfunctional immune/inflammatory response in the development of the disease [1]. First, perivascular inflammation has been frequently observed in patients with idiopathic PAH (IPAH) [10] and PAH associated with connective tissue disease (PAH-CTD) [11, 12]. Second, perivascular lesions are characterised by varying levels of infiltrating immune cells [13]. Third, circulating levels of cytokines and chemokines are increased in PAH patients [13, 14], which may lead to pulmonary vascular remodelling through alterations in pulmonary vascular cell proliferation, migration and differentiation [13]. Fourth, clinical studies have demonstrated that combining immunosuppressive and vasodilator therapies can improve pulmonary arterial pressure, prognosis and functional class in patients with PAH associated with some forms of CTD [15, 16]. As a result of these observations, the efficacy and safety of several immunomodulatory therapies are currently being assessed in clinical trials (summarised in table 1) [17–30].

Ubenimex is being developed as a potential treatment for PAH as it is an inhibitor of the inflammatory mediator leukotriene B4 found in the serum of some PAH patients [31]. A phase II trial and subsequent open-label extension study are currently planned to assess the efficacy and safety of ubenimex in PAH [17, 18]. Rituximab is a monoclonal antibody which can target the $CD20^+$ B-cells found in plexiform lesions in PAH [32, 33]. Based on the results of case reports of improved outcomes in patients with advanced PAH associated with systemic sclerosis (PAH-SSc) [33], this drug is currently being investigated in an ongoing phase II trial in PAH-SSc patients [19]. Cytokines such as interleukin (IL)-6 have been linked to the pathogenesis of PAH through their ability to control cell migration, differentiation and proliferation [13]. As a result, tocilizumab (a monoclonal antibody to the IL-6 receptor) is currently being investigated in an open-label phase II trial in PAH patients [20].

Therapies targeting mitochondrial dysfunction

Impaired mitochondrial function may lead to vascular remodelling and thus contribute to the pathogenesis of PAH [34]. Therefore, drugs that may target mitochondrial dysfunction, such as bardoxolone methyl (an Nrf2 activator and inhibitor of the NF-κB pathway) and GS-4997 (an inhibitor of apoptosis signal regulating kinase 1), are being investigated as potential PAH therapies (summarised in table 1). Promising results were obtained for bardoxolone methyl in a phase II study in PAH patients on background therapy [21] and consequently a large phase III trial is now planned in a population of patients with PAH-CTD [22]. GS-4997 is also currently under investigation in an ongoing phase II trial in PAH patients [23].

Therapies targeting BMPR2 signalling

The critical role played by the BMPR2 pathway in the pathogenesis of PAH is illustrated by the fact that >80% of patients with heritable PAH and ~20% of patients with sporadic IPAH have germline mutations resulting in loss of BMPR2 function [35, 36]. Furthermore, reduced expression of BMPR2 has also been reported in PAH patients without a mutation in this gene [37]. Thus, there is a clear rationale for activating BMPR2 signalling as a treatment option for PAH [38]. In order to identify an activator of BMPR2 signalling, a high-throughput screen of >3500 US Food and Drug Administration-approved drugs was performed [39]. Tacrolimus (a calcineurin inhibitor) was identified and subsequently demonstrated to reverse pulmonary hypertension in animal models. As a result, a phase II trial investigating the safety and efficacy of tacrolimus was initiated, but was ultimately terminated due to funding and recruitment issues (summarised in table 1) [24]. A follow-up phase IIb efficacy trial is planned [24].

Therapies targeting iron deficiency

There is evidence that iron homeostasis is important in PAH, with studies indicating that iron deficiency is common in PAH patients [40–43], and is associated with reduced exercise capacity [41, 42] and increased mortality [44]. Worsening iron deficiency has been shown to correlate with increased disease

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TABLE 1 Potential pulmonary arterial hypertension (PAH) therapies currently in development

Therapy	Clinical trial identifier	Clinical trial design	Primary end-points	Treatment duration	Status (October 2016)
Therapies targeting inflammation and immunity					
Ubenimex	NCT02664558	Phase II, multicentre, randomised, double-blind, placebo-controlled trial in PAH patients	Change in PVR	24 weeks	Recruiting [17]
	NCT02736149	Phase II, open-label, multicentre, extension study in PAH patients	Frequency of adverse events	~1 year	Not yet recruiting [18]
Rituximab	NCT01086540	Phase II, randomised, double-blind, placebo-controlled trial in PAH-SSc patients	Change in PVR	24 weeks	Recruiting [19]
Tocilizumab	NCT02676947	Phase II, open-label trial in PAH patients	Incidence and severity of adverse events; change in PVR	6 months	Recruiting [20]
Therapies targeting mitochondrial dysfunction					
Bardoxolone methyl	NCT02036970	Phase II, double-blind, randomised, interventional trial in pulmonary hypertension Group I, II or V patients	Change in 6MWD	16 weeks	Preliminary results published [21]
	NCT02657356	Phase III, double-blind, early interventional trial in PAH-CTD patients	Change in 6MWD	24 weeks	Recruiting [22]
GS-4997	NCT02234141	Phase II, dose-ranging, randomised, double-blind, placebo-controlled trial in PAH patients	Change in PVR	24 weeks	Ongoing, not recruiting [23]
Therapies targeting BMPR2 signalling					
Tacrolimus	NCT01647945	Phase II, double-blind, randomised trial in PAH patients	Frequency of adverse events	16 weeks	Terminated due to limited funding/slow patient recruitment; follow-up multicentre phase IIb efficacy trial planned [24]

Continued

TABLE 1 Continued

Therapy	Clinical trial identifier	Clinical trial design	Primary end-points	Treatment duration	Status (October 2016)
Therapies targeting iron deficiency					
Ferinject (ferric carboxymaltose)	NCT01447628	Phase II, double-blind, randomised, interventional trial in IPAH, HPAH and anorexigen-associated PAH patients	Change in PVR and exercise capacity	24 weeks	Recruiting [25]
	NCT01847352	Single-blind, nonrandomised, interventional, trial in healthy volunteers who met iron-deficient or iron-replete criteria	Change in PASP following *i.v.* iron infusion	1 week	Completed: April 2014 [26, 27]
Ferrous sulfate (oral dietary iron supplement)	NCT01446848	Interventional, open-label study in IPAH patients with iron deficiency	Change in zinc protoporphyrin level; change in serum ferritin level	12 weeks	Completed: August 2014 [28]
Pulmonary artery denervation					
Pulmonary arterial denervation procedure	chiCTR-ONC-12002085	Phase II, observational, unblinded, nonrandomised study in PAH and PAH-CTD patients	Change in PASP and 6MWD	24 weeks	Completed: April 2014 [29]
	NCT02525926	Single-blind, randomised, interventional efficacy study in PAH patients	Mean pulmonary artery pressure	26 weeks	Recruiting [30]

PVR: pulmonary vascular resistance (as measured by right heart catheterisation); PAH-SSc: PAH associated with systemic sclerosis; 6MWD: 6-min walk distance; PAH-CTD: PAH associated with connective tissue disease; BMPR2: bone morphogenetic protein receptor type 2; IPAH: idiopathic PAH; HPAH: heritable PAH; PASP: pulmonary artery systolic pressure.

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severity, as assessed by increased mean pulmonary artery pressure, reduced cardiac index and worsening functional class in IPAH patients [40]. Iron deficiency is also a finding in patients with heart failure, and likewise is associated with poor survival [45] and reduced exercise capacity [46]. This deficit in exercise capacity can be reversed by iron supplementation [46]. These findings provide a rationale for therapeutic interventions to address iron deficiency in PAH patients. To date, the clinical benefit of iron infusions in PAH patients has been demonstrated in two small exploratory studies [47, 48]. Although the results of these small studies are promising, it should be noted that low serum iron may offer a protective role in the development of pulmonary hypertension [49]. Thus, randomised controlled trials of iron supplementation in PAH patients are warranted to ensure that the impact of addressing iron deficiency in PAH can be fully elucidated.

Clinical trials investigating the efficacy of iron administration in PAH patients and healthy volunteers are summarised in table 1. A phase II trial is currently recruiting PAH patients to investigate the effect of an intravenous (*i.v.*) iron formulation, Ferinject (ferric carboxymaltose), on pulmonary vascular resistance (PVR) and exercise capacity [25]. Results from this trial are expected in December 2017. Ferinject was also investigated in a single-blind, interventional trial in iron-deficient or iron-replete healthy volunteers [26]. Ferinject prevented exaggerations in the hypertensive response to hypoxic exposure in iron-deficient, healthy volunteers [27]. An interventional, open-label study in IPAH patients investigated whether ferrous sulfate (oral dietary iron supplement) can improve clinical symptoms of PAH and relieve iron deficiency over 12 weeks [28]. No results have yet been reported for the latter two trials.

Pulmonary artery denervation

The neurohormonal axis is a potential therapeutic target in PAH as several studies have reported that plasma norepinephrine, muscle sympathetic nerve activity and the number of vessel sympathetic nerve endings are all elevated in IPAH patients [50]. Furthermore, it has been demonstrated in experimental models of pulmonary hypertension that disruption of sympathetic nerve fibres can improve pulmonary haemodynamics [50, 51]. The process of PADN involves ablating the nerves regulating sympathetic tone in the pulmonary artery, disrupting the nonvagal pulmonary baroreceptor reflex [50–52]. Results from the use of this technique in early clinical trials are preliminary, with a single patient case study [53] and a small proof-of-concept study demonstrating that PADN may result in improvements in 6-min walk distance (6MWD) and haemodynamic parameters in IPAH patients [52]. Although there were limitations associated with the design and analysis of the latter study [52, 54], the PADN procedure has been investigated in a phase II clinical trial, as described in table 1 [29]. However, it should be noted that this trial may be subject to similar design and analysis limitations as the earlier proof-of-concept study [55]. Another ongoing European multicentre study is investigating the efficacy of the PADN procedure in patients with PAH [30]. In all likelihood, the immediate changes in pulmonary artery pressure observed in pre-clinical and clinical studies of PADN cannot be explained by altered remodelling of the distal pulmonary arteries [50] and may result from improved pulmonary arterial compliance.

Clinical trial design in novel therapy development in PAH

Evolution of clinical trial design

Over the last two decades, the number of patients enrolled in PAH trials has risen significantly despite PAH being a rare disease. Only 81 patients were included in the pivotal randomised controlled trial in 1996 investigating epoprostenol, the first approved drug for the treatment of PAH. This trial utilised change in 6MWD from baseline to week 12 as its primary end-point [56]. Subsequent phase II/III PAH randomised controlled trials, which investigated the efficacy of a number of different PAH therapies, also used 6MWD as the primary end-point and included up to 350 patients [57–61]. Since these early trials, there has been an evolution to larger, event-driven studies [2–4, 62], with 1156 patients enrolled in the recently completed phase III GRIPHON trial [4]. From the first pivotal trial investigating epoprostenol in 1996, through to the GRIPHON trial in 2015, the evolving trial design in PAH has meant that the number of patients required for a phase III trial is now higher than ever. Therefore, important considerations must be taken into account when developing new drugs for PAH to reliably evaluate the preliminary efficacy, safety and the optimal dose range for the candidate drug prior to progressing to phase III trials.

Pre-clinical studies

The first step in drug development is the generation of pre-clinical data, which can provide a rationale for subsequent clinical development. However, a limitation of these pre-clinical studies is a possible lack of reproducibility, with one analysis suggesting that almost 80% of pre-clinical research data could not be reproduced [63]. This highlights the importance of using standardised techniques to ensure that data are as robust as possible. Animal models, while providing significant insights into the molecular and cellular pathways that underlie the development of PAH [64], are also subject to inherent limitations, including

immutable species differences and the inability to completely replicate human heart failure causes and manifestations [65]. While animal models remain essential for pre-clinical drug development, the currently available acute models of pulmonary hypertension are not ideal as they do not fully represent the nature of the chronic disease in humans [64].

There is some indication that cells derived from PAH patients show increased inflammatory indices [66] and a hyperproliferative and apoptosis-resistant phenotype [67] compared with control cells. *Ex vivo* studies using these cells may potentially be useful in future studies as a "first-step" test of novel compounds [68]. Such *ex vivo* studies have revealed clinically important information regarding the manifestation of PAH in humans, which cannot be modelled in animals, including transcriptional, genetic and epigenetic pathogenic markers. It has also been suggested that drug efficacy in future pre-clinical studies should be demonstrated in blinded, randomised studies using relevant end-points such as changes in PVR and right ventricular function. These changes to pre-clinical study design would strengthen the evidence supporting the progression to clinical trials. However, even with the implementation of these changes, pre-clinical studies cannot demonstrate the whole-body effects of novel compounds in humans. In order to obtain this information, clinical trials remain essential.

Early clinical trial considerations

There is an obligation to terminate the development of ineffective drugs to reduce the number of patients exposed to them. Early-phase clinical trials are an essential bridge between pre-clinical research and large-scale human studies. Careful planning and execution of early clinical trials in PAH is important. Phase I trials are designed to assess the safety, tolerability, dose, pharmacokinetics and pharmacodynamics of the clinical candidate for administration in humans, before progressing to phase II clinical trials. Phase II or "proof-of-concept" trials, although smaller and less powerful than phase III trials, can generate critical information regarding dosing and safety in patients and can provide insight into whether a large phase III trial is likely to be successful [69, 70]. However, there are limitations associated with phase II trials in PAH, including small sample sizes, heterogeneity of study populations and the selection of appropriate end-points [69]. As a result, careful planning is warranted for phase II trials. In addition to providing safety data, phase II studies could include functional capacity and clinical worsening events, as well as N-terminal pro-brain natriuretic peptide and haemodynamic variables, such as PVR, cardiac index and right atrial pressure.

Despite strong pre-clinical data, candidates might not show efficacy in PAH clinical trials, as observed in studies of selective serotonin reuptake inhibitors (SSRIs). SSRIs were found to attenuate and even reverse the development of pulmonary hypertension induced by chronic hypoxia or monocrotaline in animal models [71]. However, one nested case–control study with SSRIs failed to show clinical efficacy [72], and results from a phase II trial suggested that SSRI use was associated with increased mortality and clinical worsening [73]. For therapies with a novel mechanism of action in a particular indication, it is critical that phase II studies are performed in the relevant patient population. This is demonstrated using the example of the tyrosine kinase inhibitor nilotinib. Despite being approved in chronic myelogenous leukaemia and preventing angioproliferation in a rodent model of pulmonary hypertension with systemic sclerosis [74], nilotinib has not progressed beyond phase II in PAH. A phase II trial of nilotinib in patients with PAH was terminated due to serious adverse events in this population [71].

Phase III clinical trial considerations

Phase III PAH trials require both high numbers of patients and significant resources. Therefore, it is important that data from pre-clinical studies and phase I and II trials are as robust and reliable as possible before initiating phase III studies, particularly when testing novel therapies that act upon putative disease targets. The value of obtaining consistent phase II data, ahead of progressing to phase III trials, is illustrated by the experience with the tyrosine kinase inhibitor imatinib. This drug was investigated in a phase III trial (enrolling 202 patients with PAH) [75] and subsequent open-label extension [76], although results from an earlier phase II study in 59 patients with PAH had been inconsistent with respect to efficacy end-points [77]. In the phase III study (IMPRES), imatinib treatment resulted in significant improvements in 6MWD (primary end-point) and also led to improvements in a number of haemodynamic parameters [75]. However, imatinib failed to delay the time to clinical worsening (composite secondary efficacy end-point), and was associated with a high incidence of drug discontinuation and a higher than expected incidence of subdural haematoma in the open-label extension [75, 76]. Consequently, the benefit/risk ratio was deemed unfavourable and the manufacturer withdrew its application for a licence for imatinib for PAH. The IMPRES study therefore highlights the increasing importance of using appropriate outcome-based end-points in PAH trials to ensure that clinically relevant improvements in patient outcomes can be determined, and the importance of collecting relatively long-term safety data. Prior to the phase III trials with macitentan and selexipag, PAH therapies had been granted regulatory approval based on the results of short-term studies

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measuring exercise capacity [56–61, 78]. However, with these more recent trials there has been a shift towards the use of a composite morbidity and mortality end-point [3, 4, 62], which is now recommended as the primary end-point in pivotal phase III PAH trials [79]. Recommended components of such a primary end-point include death, lung transplantation, PAH-related hospitalisation, initiation of parenteral prostacyclin analogue therapy, disease progression (measured by a decrease in functional class and exercise capacity) and worsening of PAH symptoms (including dyspnoea, chest pain, dizziness/syncope and fatigue) [69, 79]. To ensure the robustness of the data obtained from a composite end-point, all events should be adjudicated by a blinded committee to confirm that they meet the criteria set out in the protocol. Although it is desirable that the components of a composite end-point are equally weighted in terms of clinical relevance, an important consideration is the size of the patient population to be investigated. In a rare disease such as PAH, limiting the number of end-point components to include only those with similar clinical significance could reduce the number of events obtained. This in turn could lead to a decrease in the sensitivity or statistical power of the trial in question. The integrity of the composite end-point is dictated by each and every component, and as a result the more subjective component of disease progression must be both robust and well-defined. This is particularly important given that, due to the progressive nature of the disease, this component occurred more frequently than harder components such as death or initiation of *i.v.* prostacyclin therapy in several studies performed to date [3, 4]. A number of long-term studies have been completed recently using variations of a composite primary end-point, including SERAPHIN [3], GRIPHON [4], COMPASS-2 [62] and AMBITION [2]. Three of these studies met their primary end-point over an observation period of 15–27 months; however, for each one the treatment effect was evident at 12 months [2–4]. This observation should be taken into account for future studies in PAH. Looking ahead there may also be a move towards the use of a primary composite end-point that reflects disease improvement rather than worsening, as one important goal of PAH therapy is the clinical improvement of patients. Such an end-point may be of particular relevance for future studies that evaluate the comparative benefits of different treatment regimens.

Conclusion

Significant progress has been made in the treatment of PAH through the development of drugs that target three key pathophysiological pathways, although management remains suboptimal for many patients. It is important that research continues into novel treatments in PAH to further improve outcomes for patients. Such research should extend to investigating targets beyond the signalling pathways already subject to pharmacological intervention. Furthermore, evolving pre-clinical studies and clinical trial designs hold promise for the continued development of treatment strategies in PAH.

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