




# Clinical trial design and new therapies for pulmonary arterial hypertension

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**State of the art and research perspectives in clinical trial design and new therapies for pulmonary arterial hypertension** <http://ow.ly/VHQ030mfRxc>

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**ABSTRACT** Until 20 years ago the treatment of pulmonary arterial hypertension (PAH) was based on case reports and small series, and was largely ineffectual. As a deeper understanding of the pathogenesis and pathophysiology of PAH evolved over the subsequent two decades, coupled with epidemiological studies defining the clinical and demographic characteristics of the condition, a renewed interest in treatment development emerged through collaborations between international experts, industry and regulatory agencies. These efforts led to the performance of robust, high-quality clinical trials of novel therapies that targeted putative pathogenic pathways, leading to the approval of more than 10 novel therapies that have beneficially impacted both the quality and duration of life. However, our understanding of PAH remains incomplete and there is no cure. Accordingly, efforts are now focused on identifying novel pathogenic pathways that may be targeted, and applying more rigorous clinical trial designs to better define the efficacy of these new potential treatments and their role in the management scheme. This article, prepared by a Task Force comprised of expert clinicians, trialists and regulators, summarises the current state of the art, and provides insight into the opportunities and challenges for identifying and assessing the efficacy and safety of new treatments for this challenging condition.

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## Current state of clinical trial design and therapeutics in pulmonary arterial hypertension

With advances in our understanding of the pathobiology of pulmonary hypertension (PH) over the past 20 years, more than 10 drugs have been developed and approved for the treatment of pulmonary arterial hypertension (PAH) and one for chronic thromboembolic PH (CTEPH). Initial clinical trials performed in newly diagnosed PAH and CTEPH were single agent, placebo controlled, of short duration, focused on changes in measures of exercise capacity and comprised of relatively small populations of patients. However, over the past 5 years, clinical trial designs testing novel therapies for PAH have evolved into much larger, placebo controlled, on background therapy and upfront combination therapy trials. In addition, event-driven studies examining the effect of sequential combination therapy on clinical worsening have forced the community to search for more clinically relevant, novel efficacy end-points and trial design. Here, we review the evolution of clinical trial end-points, report on new therapeutic targets, evaluate clinical trial design and propose goals for clinical investigation.

### Evolution of end-points in clinical trials of PH

#### 6-min walk test

The 6-min walk test (6MWT), a submaximal exercise test, has been the most commonly employed primary end-point in clinical trials of PH therapies, beginning with the first randomised controlled trial (RCT) for drug registration of epoprostenol in 1990 [1]. Since that initial study, most of the registration studies for novel PAH or CTEPH therapies employed short-term change in distance achieved on the 6MWT ( $\Delta$ 6MWD) as the primary outcome (figure 1) [2–13]. These studies identified statistically significant differences in  $\Delta$ 6MWD that resulted in regulatory approval for use in PH, but the clinical relevance of these changes remained less well defined. Multiple studies examining the relationship between  $\Delta$ 6MWD and short- and long-term outcomes, such as need for hospitalisation, lung transplantation, initiation of rescue therapy or death, failed to consistently demonstrate significant associations [14–18]. Subsequent studies defined clinically relevant changes in 6MWD pertaining to patient-important outcomes, such as health-related quality of life and prediction of clinical deterioration [17, 19–21]. However, the utility of  $\Delta$ 6MWD as a primary outcome measure in clinical trials is limited, particularly in

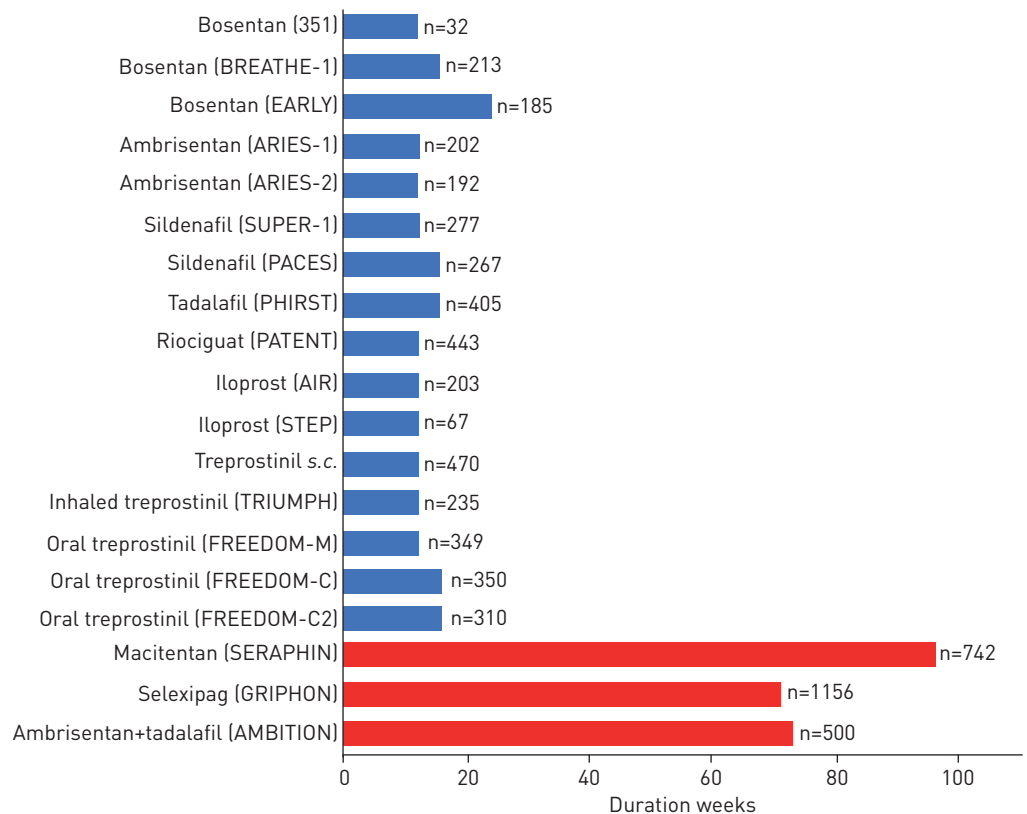


FIGURE 1 Duration of main registration studies (randomised controlled trials (RCTs)) for currently approved pulmonary arterial hypertension therapies. Blue bars: RCTs with change in 6-min walk distance as primary outcome measure; red bars: RCTs with morbidity and mortality composite primary outcome measure.

more contemporary trials involving sequential, add-on therapy. The change is less than the clinically relevant thresholds described, despite the significance achieved by other clinical outcomes [7–10, 22, 23].

#### *Patient-reported outcomes*

Alternate outcome measures that are clinically meaningful end-points, measuring how a patient “feels, functions or survives”, are sought by regulatory agencies. In PH, patient-reported outcomes (PROs) now exist, but have been less responsive to therapeutic impact [24–26]. Disease-specific measures need validation in varied languages and need to be included in future clinical trials at all stages of development. The CAMPHOR (Cambridge Pulmonary Hypertension Outcome Review) questionnaire, the first PAH/CTEPH-specific questionnaire, is quite lengthy and does not track with other clinical measures over time [27]. The 10-question survey proposed by the Pulmonary Hypertension Association UK (emPHasis-10) is more efficient, but needs further study [28], and the recently reported SYMPACT study appears to be the more efficient and inclusive, but also needs additional validation [29].

#### *Other surrogate end-points*

PH is a disease that lacks strong surrogate end-points [30]. By definition, a surrogate end-point should be: 1) part of the causative pathway from therapy to clinical outcome, 2) its baseline value should be related to clinical outcome, 3) a change in its value should reflect a change in outcome both in direction and magnitude of change, and 4) the estimate of its clinical benefit should be independent of the nature of treatment (*e.g.* the relationship of change in the surrogate with the outcome should be the same regardless of the intervention that led to the change) [31]. A recent systematic review of surrogate end-points employed in clinical trials of PAH therapy between 1985 and 2013 found that inclusion of invasive measures, such as haemodynamics, as either primary or secondary end-points, significantly decreased over time, while measures of functional capacity, functional status and PROs increased significantly [32].

#### *Evolution to composite outcome end-points*

To address some of the limitations of the 6MWT as a primary outcome measure in clinical trials and to explore novel end-points, registration studies in the mid-2000s incorporated composite end-points reflecting time to clinical worsening (TTCW) [6, 7]. Reductions in risk of clinical deterioration noted in these studies motivated the decision to use this composite end-point of morbidity and mortality as the primary outcome in a large registration study of macitentan [22]. The significant reduction in the risk of clinical worsening noted between treatment arms was not reflected by the  $\Delta$ 6MWD. This suggested a distinct effect on the risk of clinical events *versus* improvement of functional capacity. Similar associations between treatment assignment, TTCW and  $\Delta$ 6MWD were noted in the RCT of selexipag [23]. Combined with data from an RCT of initial combination of ambrisentan and tadalafil *versus* either one alone demonstrating responsiveness of the measure in a treatment-naïve cohort, TTCW is now an approved end-point for PAH therapeutic registration trials (ClinicalTrials.gov identifiers NCT01908699, NCT02932410 and NCT01824290) (table 1).

#### *Limitations to outcome end-points*

Future clinical trials need to be more efficient. While TTCW as the primary end-point has been widely accepted, it is not the solution. First, there is no standard definition across trials for TTCW, limiting the ability to compare treatment effects between studies (table 1). Second, each component of a composite end-point should be weighted with respect to clinical importance and frequency of occurrence; the current components of TTCW in PH are not (*e.g.* death is infrequent and clinical worsening is frequent) [33]. Third, the relationship between clinical worsening and subsequent survival is not well defined and not always adjudicated by an events committee with expertise in PH (table 1) [34]. A more recent analysis utilising data from the SERAPHIN [22] and GRIPHON [23] TTCW trials found a strong association between both symptomatic progression and hospitalisation and mortality at 3, 6 and 12 months [35]. Rigorous studies of the association between TTCW and subsequent events, such as survival, are still needed to fully establish TTCW as a validated surrogate for mortality.

### **New drug targets for PAH**

The currently licensed drugs are directed at correcting the imbalance of vasoactive factors in PAH. There is widespread acceptance that new drugs need to address other pathological mechanisms that drive vascular remodelling. There is no shortage of novel drug targets of potential therapeutic interest; the current challenge is prioritising candidates according to likelihood of success in clinical trials, side-effects, quality of life and cost-effectiveness.

#### *Genetically determined targets*

Genetics is a powerful mechanism for identifying and prioritising new drug targets with confidence. Mutations in *BMPR2* (bone morphogenetic protein receptor type 2), the most common susceptibility gene

TABLE 1 Definitions of time to clinical worsening (TTCW) when used as primary outcome measure in pulmonary arterial hypertension (PAH) clinical trials

Macitentan <i>versus</i> placebo (SERAPHIN) [22]	Selexipag <i>versus</i> placebo (GRIPHON) [23]	Ambrisentan+tadalafil <i>versus</i> either alone (AMBITION) [102]
Worsening PH: ≥15% decrease in 6MWD from baseline + Worsening symptoms (WHO FC or signs of right heart failure) + Need for additional PAH therapy	Disease progression: ≥15% decrease in 6MWD from baseline + Worsening WHO FC or need for additional PAH therapy	Disease progression: ≥15% decrease in 6MWD from baseline + WHO FC III or IV
	Hospitalisation for worsening PAH	Hospitalisation for worsening PAH
Start of <i>i.v./s.c.</i> prostanoids therapy	Start of <i>i.v./s.c.</i> prostanoids therapy or LTOT	Start of <i>i.v./s.c.</i> prostanoids therapy
Lung transplantation or atrial septostomy	Lung transplantation or atrial septostomy	Lung transplantation or atrial septostomy
		Unsatisfactory long-term clinical response: Any decrease in baseline 6MWD at two consecutive visits + WHO FC III at two visits separated by at least 6 months
All-cause death	All-cause death	All-cause death

PH: pulmonary hypertension; 6MWD: 6-min walk distance; WHO: World Health Organization; FC: functional class; LTOT: long-term oxygen therapy.

for PAH, have focused attention on the transforming growth factor- $\beta$  (TGF- $\beta$ )/BMP signalling pathway [36]. To date, the only treatment that has been used to target BMPR2 signalling in clinical trials is FK506 (tacrolimus) [37], which is currently in phase 2 (ClinicalTrials.gov identifier NCT01647945). FK506 binds its pharmacological target FKBP12 (12-kDa FK506-binding protein) and removes it from all three BMPR type 1 receptors (ALK1, ALK2 and ALK3), including those preferred by BMPR2 (ALK1 and ALK3). It is able to activate BMPR2-mediated signalling even in the absence of exogenous ligand and BMPR2.

Strategies proposed for correcting impaired BMPR2 signalling, beyond the aspiration of gene therapy, include pharmacological approaches such as chloroquine (which prevents lysosomal degradation of the BMPR2), ataluren (with the aim of reading through missense mutations) and increasing BMP9 levels [38]. An alternative to increasing BMP signalling is to inhibit TGF- $\beta$  activity using a novel activin-receptor fusion protein (sotatercept) that competitively binds and neutralises TGF- $\beta$  superfamily ligands [39]. Potential targets will emerge as we better understand the genetic architecture of PAH, including ion channels (*KCNK3* (potassium channel subfamily K, member 3)), aquaporin and *SOX17* (SRY-box 17) [36, 40]. Further pre-clinical studies will be needed to realise how these, or their signalling pathways, might be manipulated for therapeutic benefit.

#### Epigenetic modification

Little is known of the epigenetics of PAH, but studies of histone deacetylase (HDAC) inhibitors in pre-clinical cell and animal models have reported beneficial effects, although cardiotoxicity is a concern [41]. Translating the therapeutic potential of HDAC inhibition in PAH will require a clearer insight into the HDAC subtypes involved in the vascular pathology. Likewise, a better understanding of the role of microRNAs in PAH is needed to inform how best to manipulate pharmacologically to have an impact on pulmonary vascular disease [42].

#### DNA damage

Inhibition of poly(ADP-ribose) polymerase (PARP) reverses PAH in several animal models [43]. An open-label early phase 1 study of olaparib, an orally available PARP1 inhibitor approved for the treatment of *BRCA* (breast cancer gene)-related breast cancer, has been proposed in PAH patients in World Health Organization Functional Class II–III on stable vasoactive therapy (ClinicalTrials.gov identifier NCT03251872).

### **Growth factors**

The tyrosine kinase receptor imatinib was the first significant compound used in a large-scale trial to directly target vascular remodelling in PAH. Imatinib inhibits platelet-derived growth factor (PDGF) receptor- $\alpha$  and - $\beta$  [44]. PDGF is a trophic factor in vascular cells and lung tissue from PAH patients shows increased expression of PDGF receptors. The phase 3 PAH trial reported a 32 m increase in mean placebo-corrected 6MWD and a reduction in pulmonary vascular resistance (PVR) without improvement in TTCW. Unfortunately subdural haematoma occurred in eight patients receiving both imatinib and anticoagulation with vitamin K antagonists [45]. However, there remains considerable interest in tyrosine kinase inhibitors, and imatinib in particular, as some patients have stabilised and even improved on imatinib treatment in anecdotal reports. Identifying the clinical and molecular characteristics of patients likely to respond is key to the success of this programme.

Clinical experience with other tyrosine kinase inhibitors urges caution. Indeed, exposure to dasatinib is associated with the development of PAH [46, 47]. In a 16-week, single-centre, open-label trial in 12 patients with PAH, the multikinase/angiogenesis inhibitor sorafenib worsened pulmonary haemodynamics and was associated with adverse events, such as moderate skin reactions on the hands and feet and alopecia [48].

### **Metabolism**

Insulin resistance contributes to the morbidity and mortality of PAH [49]. This has led to interest in the use of agents such as rosiglitazone, metformin and glucagon-like peptide 1 agonists (table 2) [50, 51]. A small clinical trial with metformin is underway, with detailed study of the impact on the right ventricle and pulmonary haemodynamics. Whereas normal myocardium uses fatty acid oxidation as an energy source, the maladapted myocardium relies on glycolysis, which is much less efficient [52]. Glutaminolysis is also induced. Inhibition of fatty acid oxidation increases glucose oxidation, leading to the possibility that trimetazidine (an inhibitor of 3-ketoacyl coenzyme A thiolase), approved for angina, may have utility in PAH [53]. Ranolazine, another antianginal agent, is also an option [54].

In PAH, proliferating cells switch their metabolism from oxidative phosphorylation to glycolysis for ATP production. Dichloroacetate (DCA), which inhibits pyruvate dehydrogenase kinase, is a potential treatment for PAH [55]. Gene variants that reduce the function of SIRT (sirtuin 33) or UCP2 (uncoupling protein 2) impair the clinical response to DCA. A small clinical study with DCA has shown haemodynamic improvement in genetically susceptible patients [56].

### **Inflammation and immune modulation**

Histological studies of the lung, the presence of circulating autoantibodies and high plasma levels of cytokines in PAH all point to inflammation as a driver of its pathology [57]. Direct investigation of this currently lies with a clinical trial of the anti-CD20 monoclonal antibody, rituximab, in PAH associated with connective tissue disease (ClinicalTrials.gov identifier NCT01086540), and a trial of tocilizumab, an interleukin-6 receptor antagonist in PAH (ClinicalTrials.gov identifier NCT02676947) (table 3). Elafin, an endogenously produced elastase inhibitor, has now progressed to clinical trials [58]. Elafin is pro-apoptotic and decreases neointimal lesions in lung organ culture [59].

### **Oestrogen signalling**

Aromatase converts androgens to oestrogen and is evident in the pulmonary vasculature of PAH lungs. A small trial of anastrozole, an aromatase inhibitor, in PAH has reported an increase in 6MWD [60] and a further study is underway (table 4).

### **Oxidative and hypoxic stress**

Oxidative stress is another mechanism that has been targeted in PAH. In contrast to animal studies [61], a phase 2 study of an inhibitor of apoptosis signal-regulating kinase 1 (ASK1) failed to show overall improvement [62]. A phase 3 study with bardoxolone methyl in scleroderma has been completed, following some signals of efficacy in phase 2 (ClinicalTrials.gov identifier NCT02036970). This drug induces the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates antioxidant proteins, and suppresses activation of the pro-inflammatory factor NF- $\kappa$ B.

Increased expression of hypoxia-inducible factor (HIF), specifically HIF1 $\alpha$  and HIF2 $\alpha$ , in the PAH lung, genetic manipulation in animals [63] and naturally occurring mutations in humans (e.g. Chuvash polycythaemia [64]) highlight the potential importance of hypoxic stress in PAH. Several groups have shown that iron deficiency in the absence of anaemia is common in PAH and is associated with reduced survival in PAH [65]. Open-label studies of *i.v.* iron replacement in PAH suggest an improvement in exercise capacity [66] and a double-blind study is near completion.

TABLE 2 Clinical trials with drugs targeting metabolic dysfunction in pulmonary arterial hypertension (PAH)

Drug tested	ClinicalTrials.gov identifier	Study design	Study duration	Main inclusion criteria	Primary outcome measure	Secondary outcome measures
<b>Fatty acid oxidation: ranolazine and trimetazidine</b>						
Ranolazine	NCT02829034	Multicentre RCT <i>versus</i> placebo	26 weeks	PH on stable specific therapies but with RV dysfunction (RVEF <45%)	Percentage change in RVEF (assessed by MRI)	
Ranolazine	NCT01839110	Multicentre RCT <i>versus</i> placebo	26 weeks	PH on stable specific therapies but with RV dysfunction (RVEF <45%)	Number and percentage of subjects with high-risk profile at end of the study	Baseline comparison of the metabolic profiling, microRNA and iPSCs of subjects with and without RV dysfunction
Ranolazine	NCT02133352	Single-centre open-label phase 4	26 weeks	PH with LV diastolic dysfunction	Percentage change in mPAP, PAOP and PVR (RHC)	Other haemodynamic variables, 6MWD, MRI, echocardiography and NT-proBNP
Ranolazine	NCT01757808	Phase 1	12 weeks	PAH on one or more background specific therapies (including <i>i.v./s.c.</i> prostacyclins)	Change in PVR (RHC)	Change in CPET, RV echocardiography parameters and 6MWD
Trimetazidine	NCT03273387	Single-centre phase 2 and 3	12 weeks	PAH	Changes in RV function (MRI)	Changes in cardiac fibrosis level (MRI), NYHA FC and LDH level
<b>Glycolysis: dichloroacetate</b>						
Dichloroacetate	NCT01083524	Two-centre open-label phase 1	16 weeks	PAH on one or more background oral specific therapies	Safety and tolerability	Change in PVR, 6MWD, RV size and function (MRI), NT-proBNP and lung/RV metabolism (FDG-PET)
<b>Modulation of Nrf2 pathway/NF-κB pathway: bardoxolone methyl</b>						
Bardoxolone methyl	NCT02036970 (LARIAT)	Multicentre phase 2 RCT <i>versus</i> placebo	16 weeks	PAH, PH-ILD, subset of patients with group 3 or 5 PH	Change in 6MWD	Determine recommended dose range
Bardoxolone methyl	NCT02657356 (CATALYST)	Multicentre phase 3 RCT <i>versus</i> placebo	24 weeks	PAH-CTD	Change in 6MWD	
Bardoxolone methyl	NCT03068130 (RANGER)	Multicentre phase 3 open-label extension	Up to 5 years	Patients with PH who previously participated in RCTs with bardoxolone	Long-term safety	
<b>Metabolic syndrome: AMPK signalling and metformin</b>						
Hormonal, metabolic and signalling interactions in PAH	NCT01884051	Observational	5 years	PAH and healthy subjects	Safety and biomarkers of mechanism	
Metformin	NCT03349775	Early phase 1	12 weeks	PH and obesity	Pulmonary vascular haemodynamics at rest and on exercise	Effect on PA endothelial cell phenotypes

RCT: randomised controlled trial; PH: pulmonary hypertension; RV: right ventricle; RVEF: right ventricular ejection fraction; MRI: magnetic resonance imaging; iPSC: induced pluripotent stem cell; LV: left ventricle; mPAP: mean pulmonary arterial pressure; PAOP: pulmonary artery occlusion pressure; PVR: pulmonary vascular resistance; RHC: right heart catheterisation; 6MWD: 6-min walk distance; NT-proBNP: N-terminal pro-brain natriuretic peptide; CPET: cardiopulmonary exercise testing; NYHA: New York Heart Association; FC: functional class; LDH: lactate dehydrogenase; FDG: <sup>18</sup>F-2-fluoro-2-deoxy-D-glucose; PET: positron emission tomography; Nrf2: nuclear factor erythroid 2-related factor 2; ILD: interstitial lung disease; AMPK: AMP-activated protein kinase; PA: pulmonary artery.

TABLE 3 Clinical trials with drugs targeting inflammation in pulmonary arterial hypertension (PAH)

Drug tested	ClinicalTrials.gov identifier	Study design	Study duration	Main inclusion criteria	Primary outcome measures	Secondary outcome measures
<b>Modulation of cytokines pathway: anakinra and tocilizumab</b>						
Anakinra	NCT01479010	Single-centre open-label pilot study: phase 1 and 2	4 weeks	PAH (excluding PAH-CTD, ILD, POPH) in FC III despite optimal PAH therapy	Change in peak $V_{O_2}$ and in $V'E/V'CO_2$ slope (CPET)	Change in biomarkers; correlation between changes in biomarkers and CPET measures
Anakinra	NCT03057028	Single-centre open-label pilot study: phase 1	14 days	PAH (excluding PAH-CTD) in FC II or III despite optimal PAH therapy	Change in peak $V_{O_2}$ and ventilatory efficiency (CPET)	Change in hs-CRP, NT-proBNP, IL-6 and symptoms of heart failure (MLHFQ); correlation between biomarkers and measures of exercise capacity
Tocilizumab	NCT02676947	Open-label: phase 2 (TRANSFORM-UK)	24 weeks	PAH (excluding PAH-CTD due to SLE, RA and MCTD)	Safety (incidence and severity of adverse events); change in PVR (RHC)	Change in 6MWD, NT-proBNP, WHO FC and QoL
<b>Inflammation: ubenimex</b>						
Ubenimex	NCT02736149	Multicentre open-label extension study: phase 2 (LIBERTY-2)	Variable (average 1 year)	PAH on $\geq 1$ PAH-specific therapies, in FC II or III, who completed the phase 2 study	Safety (adverse events)	Change in PVR, 6MWD, WHO FC and BNP/NT-proBNP
Ubenimex	NCT02664558	Multicentre double-blind RCT versus placebo: phase 2 (LIBERTY)	24 weeks	PAH on $\geq 1$ PAH-specific therapies in WHO FC II or III	Change in PVR	Change in 6MWD, WHO FC, TTCW, QoL and NT-proBNP

CTD: connective tissue disease; ILD: interstitial lung disease; POPH: portopulmonary hypertension; FC: functional class;  $V_{O_2}$ : oxygen uptake;  $V'E/V'CO_2$ : ventilatory response (minute ventilation/carbon dioxide production); CPET: cardiopulmonary exercise testing; hs-CRP: high-sensitivity C-reactive protein; NT-proBNP: N-terminal pro-brain natriuretic peptide; IL-6: interleukin-6; MLHFQ: Minnesota Living with Heart Failure Questionnaire; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MCTD: mixed connective tissue disease; PVR: pulmonary vascular resistance; RHC: right heart catheterisation; 6MWD: 6-min walk distance; WHO: World Health Organization; QoL: quality of life; RCT: randomised controlled trial; TTCW: time to clinical worsening.

TABLE 4 Clinical trials with drugs targeting other signalling pathways

Drug tested	ClinicalTrials.gov identifier	Study design	Study duration	Main inclusion criteria	Primary outcome measures	Secondary outcome measures
<b>Modulation of the oestrogen pathway: anastrozole and fulvestrant</b>						
Anastrozole	NCT03229499	RCT <i>versus</i> placebo: phase 2 (PHANTOM)	24 weeks	PAH in WHO FC I–III; stable PAH-specific therapy allowed	Change in 6MWD	Change in 6MWD (3 and 12 months), RVEF, NT-proBNP, biomarkers, SF-36, emPHasis-10, physical activity (actigraphy), TTCW and bone mineral density
Anastrozole	NCT01545336	Double-blind RCT <i>versus</i> placebo: phase 2	12 weeks	PAH in WHO FC I–III on stable PAH-specific therapy	Change in plasma oestradiol and in TAPSE	Change in 6MWD
Fulvestrant	NCT02911844	Open-label: phase 2	9 weeks	Post-menopausal female patients with PAH in WHO FC I–III on stable PAH-specific therapy	Change in plasma oestradiol, TAPSE, 6MWD and NT-proBNP	
<b>Improvement of oxygenation: acetazolamide</b>						
Acetazolamide	NCT02755298	Double-blind crossover RCT <i>versus</i> placebo: phase 2 and 3	5 weeks	Stable patients with pre-capillary PH who are undergoing RHC for a clinical indication	Change in 6MWD	Change in QoL (MLHFQ), maximal ramp CPET, cerebral and muscle tissue oxygenation, daily activity (actigraphy), echocardiography parameters, FC, PRO (SF-36, CAMPHOR), NT-proBNP, <i>etc.</i>

RCT: randomised controlled trial; PAH: pulmonary arterial hypertension; WHO: World Health Organization; FC: functional class; 6MWD: 6-min walk distance; RVEF: right ventricular ejection fraction; NT-proBNP: N-terminal pro-brain natriuretic peptide; SF-36: Short Form-36; emPHasis-10: 10-question survey proposed by the Pulmonary Hypertension Association UK; TTCW: time to clinical worsening; TAPSE: tricuspid annular plane systolic excursion; PH: pulmonary hypertension; RHC: right heart catheterisation; QoL: quality of life; MLHFQ: Minnesota Living with Heart Failure Questionnaire; CPET: cardiopulmonary exercise testing; PRO: patient-reported outcome; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review.



### ***Serotonin and humoral modulation***

Serotonin (5-HT) is a potent vasoconstrictor and pro-proliferative factor in pulmonary vascular cells [67]. Terguride, a 5-HT<sub>2A/2B</sub> receptor antagonist, showed no clinical benefit in a phase 2 study in PAH [68]. This may be explained, in part, because the 5-HT<sub>1B</sub> receptor is the most highly expressed 5-HT receptor in the pulmonary arteries in PAH lungs. Tryptophan hydroxylase 1 (TPH1) is the rate-limiting enzyme in 5-HT biosynthesis and studies with a selective inhibitor of TPH1 are planned.

There is interest in further examination of the role of augmenting vasoactive intestinal polypeptide activity, despite previous disappointment with inhaled administration [69].

Careful use of  $\beta$ -blockers in stable PAH is safe, but which patients might benefit remains an open question [70]. While angiotensin-converting enzyme (ACE) inhibitors are importantly renoprotective in scleroderma, they have not been shown to be of benefit in PAH. The homologue of ACE, known as ACE2, converts angiotensin I and angiotensin II to angiotensin-(1-7), angiotensin-(1-9) and angiotensin-(1-5), and is of interest. Decreased ACE2 levels and ACE2 autoantibodies have been reported in PAH [71] and ACE2 replacement using a purified *i.v.* formulation of soluble recombinant human ACE2 is underway. Two prospective studies examining the effect of the aldosterone antagonist, spironolactone, are also underway.

### ***Pulmonary artery denervation***

Pulmonary artery denervation is an interventional therapy aimed at abolishing the sympathetic nerve supply to the pulmonary circulation. In a single-centre study, 13 patients underwent the procedure using a radiofrequency ablation catheter, and significant reductions of mean pulmonary arterial pressure and improvement in 6MWD were reported [72]. Several trials are ongoing to determine efficacy and safety of the procedure in PAH (table 5).

### ***Stem cells***

There are a few clinical reports of cell therapy in patients with PAH. In a 12-week open-label controlled study, infusion of autologous endothelial progenitor cells (EPCs) improved 6MWD and haemodynamic variables in adult patients with severe PAH [73]. Similar results were observed in children with idiopathic PAH [74]. In the phase 1 PHACeT trial, EPCs transduced with endothelial nitric oxide synthase were administered to patients with PAH [75]. The 6MWD improved significantly at 1, 3 and 6 months post-infusion, by 65, 48 and 47 m, respectively. During the 3-day infusion protocol, no adverse haemodynamic or gas exchange parameters were observed [75].

There are several reasons to believe that therapy with cardiac stem cells such as cardiosphere-derived cells (CDCs) would benefit PAH patients with or without right ventricular dysfunction. CDCs have been demonstrated in pre-clinical models to ameliorate most of the major aberrations that underlie the pathobiology/pathophysiology of both the maladapted right ventricle muscle and the remodelled pulmonary vessels [76]. They are potently angiogenic, antifibrotic and antiapoptotic; they have significant anti-inflammatory effects; they attenuate both oxidative and nitrosative stress; and they attract endogenous stem cells to sites of vascular injury. Recently, the phase 1 trial ALPHA of allogeneic CDCs for PAH was initiated, with the primary objective to determine the maximum feasible dose and safety profile of CDCs administered by central infusion into the right ventricle outflow tract of patients with idiopathic PAH (table 6).

### ***Clinical trial failures***

The introduction of several new drugs for the treatment of PAH speaks to the success of drug development in this therapeutic area over the past two decades, but a growing number of clinical trials have not met their primary end-point or have reported major safety concerns (table 7). Limited data are publically available on these studies, but a deeper understanding is important to reduce risk and improve success going forward.

There is a high attrition rate as drug development progresses through its phases from first-in-human studies to pivotal registration studies. Of a total of 9985 clinical trials from 7455 development programmes between 2006 and 2015, only one in 10 progressed from phase 1 to approval [77]. For cardiopulmonary disease therapeutics the range was 6.6–12.8%. Phase transition success rate ranges for cardiopulmonary diseases are estimated at 58.9–65.3% for phase 1 to 2, 24.1–29.1% for phase 2 to 3 and 55.5–71.1% for phase 3 to new drug application. Despite this poor overall probability of success, drug development for rare disease indications remains one of the more successful programmes across all phases of development [77].

The main reason for terminating the development of a drug is lack of efficacy. 52% of programmes are stopped because of lack of efficacy compared with 21% due to safety concerns [78, 79]. Our limited understanding of the pathological drivers of PH contributes to this trial failure rate. Overemphasis on a

TABLE 5 Clinical trials investigating pulmonary artery denervation

Intervention	ClinicalTrials.gov identifier	Study design	Study duration	Main inclusion criteria	Primary outcome measures	Secondary outcome measures
<b>Therapeutic Intra-Vascular UltraSound (TIVUS) system</b>	NCT02516722	Multicentre open-label study (TROPHY)	12 months	PAH in WHO FC III on stable double combination therapy other than parenteral PGI <sub>2</sub>	Safety (procedure-related AE: 1 month); safety (PAH-related AEs and all-cause death: 12 months)	Change in mPAP, PVR, 6MWD and QoL at 4 months
<b>Therapeutic Intra-Vascular UltraSound (TIVUS) system</b>	NCT02835950	Multicentre open-label study (TROPHY-US)	12 months	PAH in WHO FC III on stable double combination therapy other than parenteral PGI <sub>2</sub>	Safety (procedure-related AE: 1 month); safety (AEs, PAH-related AEs and all-cause death: 12 months)	Change in mPAP, PVR, 6MWD, QoL, NT-proBNP and RV function (MRI and echocardiography) at 6 months
<b>Pulmonary artery denervation</b>	NCT02525926	Multicentre single-blinded RCT <i>versus</i> placebo (DENERVAP)	24 weeks	PAH patients in WHO FC III-IV despite dual therapy including a PGI <sub>2</sub> or dual oral therapy in patients unable to receive PGI <sub>2</sub> therapy	Change in mPAP (RHC)	Change in mPAP (3 months), PVR and other haemodynamic variables (6 months), FC, 6MWD, oxygen dependence, supraventricular arrhythmia, BNP, cardiac troponin, and RV function (echocardiography)
<b>PA denervation (+sildenafil)</b>	NCT03282266	Multicentre single-blinded RCT <i>versus</i> placebo (PADN-CFDA)	24 weeks	PAH	Change in 6MWD	Change in haemodynamic variables (RHC), RV function (echocardiography) and PAH-related events

PAH: pulmonary arterial hypertension; WHO: World Health Organization; FC: functional class; PGI<sub>2</sub>: prostacyclin I<sub>2</sub>; AE: adverse event; mPAP: mean pulmonary arterial pressure; PVR: pulmonary vascular resistance; 6MWD: 6-min walk distance; QoL: quality of life; NT-proBNP: N-terminal pro-brain natriuretic peptide; RV: right ventricle; MRI: magnetic resonance imaging; RCT: randomised controlled trial; RHC: right heart catheterisation.

TABLE 6 Clinical trials investigating cell therapy

Cells	ClinicalTrials.gov identifier	Study design	Study duration	Main inclusion criteria	Primary outcome measures	Secondary outcome measures
<b>Autologous EPCs transfected with human eNOS</b>	NCT03001414	Multicentre double-blind crossover RCT <i>versus</i> placebo: phase 2 (SAPPHIRE)	24 weeks	PAH in WHO FC II-IV on stable PAH-specific therapy	Change in 6MWD	Change in 6MWD (3, 9 and 12 months) and PVR; number of deaths or clinical worsening of PAH; change in RV function (echocardiography and MRI) and QoL (SF-36)
<b>Allogeneic human cardiosphere-derived stem cells</b>	NCT03145298	Phase 1a: open-label; phase 1 (ALPHA) Phase 1b: double-blind RCT <i>versus</i> placebo: phase 1 (ALPHA)	1 year 1 year	PAH in WHO FC II-III on stable PAH-specific therapy	Safety (gas exchange, haemodynamics); arrhythmia; sudden death; mortality and morbidity	Long-term safety end-points; TTCW (including death)

EPC: endothelial progenitor cell; eNOS: endothelial nitric oxide synthase; RCT: randomised controlled trial; PAH: pulmonary arterial hypertension; WHO: World Health Organization; FC: functional class; 6MWD: 6-min walk distance; PVR: pulmonary vascular resistance; RV: right ventricle; MRI: magnetic resonance imaging; QoL: quality of life; SF-36: Short Form-36; TTCW: time to clinical worsening.

TABLE 7 Main recent clinical trials in pulmonary arterial hypertension with either negative result or tolerability/safety issues

Study/compound(s)	Phase	End-point: result	Formal presentation [ref.]	Published [ref.]
<b>ASA-STAT: aspirin and simvastatin</b>	2	6MWD: lack of efficacy	Yes	Yes [103]
<b>ARROW: selonsertib (ASK-1 inhibitor)</b>	2	6MWD: lack of efficacy	Yes [62]	No
<b>Cicletanine (antihypertensive with vasorelaxant and diuretic properties)</b>	2	PVR: lack of efficacy	Yes [104]	No
<b>Aviptadil (vasoactive intestinal peptide)</b>	2	PVR: lack of efficacy	Yes [105]	No
<b>IMPRES: imatinib (tyrosine kinase inhibitor)</b>	3	6MWD: positive tolerability and safety issues	Yes	Yes [45]
<b>Terguride (partial dopamine agonist and serotonin receptor antagonist)</b>	2	6MWD: lack of efficacy	Yes [68]	No
<b>LIBERTY: ubenimex (leukotriene B4 inhibitor)</b>	2	PVR: lack of efficacy	No	No

6MWD: 6-min walk distance; ASK1: apoptosis signal-regulating kinase 1; PVR: pulmonary vascular resistance.

single mechanism and the limitations of model systems also constrain drug development [78, 80]. Pre-clinical animal models do not completely reflect true human disease. When tested in the best models, novel therapies have not been evaluated in animals on background PAH therapies. Moreover, pre-clinical studies do not employ rigours such as sample size determination and randomisation, and they have not traditionally evaluated end-points used in human trials. Finally, it is possible that therapeutic responses result from off-target effects (*i.e.* enhanced blood flow to skeletal muscle), which are usually not evaluated in animal models [78, 80]. Overcoming these potential pre-clinical factors will require a more complete understanding of relevant mechanisms in modern day phenotypes through initiatives such as PVDOMICS (ClinicalTrials.gov identifier NCT02980887), and advancement of technologies to improve applicability of *ex vivo* and *in vitro* assays. Establishing rigorous standards guiding pre-clinical animal model testing [80] will also likely result in improved selection of therapeutic candidates and minimise the impact of bias and false-positive results when tested in the human clinical trial environment.

Given that clinical trials are more likely to “fail” than succeed, it is important to build in plans to learn from “failure” [81]. The goals and objectives of early-phase clinical trials should be focused on safety, informative biomarkers and identification of responsive subpopulations. While bound by statistical norms, decisions in early-phase trials should not be made based on single p-value thresholds. Basing scientific, business or policy decisions in early development on a “mechanical bright-line rule” such as p-value <0.05 as a threshold of scientific claim justification can lead to incorrect conclusions [82]. Later-phase studies need to be informed by adequate data on dose–response relationships and the characteristics of patients that show an efficacy signal. However, data are generated all along the drug development pathway and it is imperative that these data are available for careful evaluation. This will inform critical analysis of study power, end-points and population selection.

Although commonly used, the term “failure” in the context of clinical trials does not distinguish between well-designed negative studies *versus* studies that are simply inconclusive due to flawed design or conduct. Moreover, the perception of clinical trial “failure” is complex and depends on perspective. While clinical trialists and scientists may consider interventions that do not meet relevant primary or secondary end-points as “failed”, patients may think of therapies that do not provide symptomatic improvement as unhelpful. Beyond issues of definition and perspective, data from these clinical trials are often not publically disclosed due to a multifaceted set of issues ranging from business agendas and academic disinterest to publication bias. Unsuccessful trials can be as, or even more, informative as “positive” trials. Data from the IMPRES trial serve as a good example [45]. Despite demonstrable improvement in exercise capacity and haemodynamics, the higher rate of serious adverse events and study drug discontinuation ultimately led to the decision to halt development of imatinib mesylate as add-on therapy for PAH. However, the publication and ensuing academic dialogue has empowered discussion around the methodology, dosing and approach to repurposing of therapeutics in PAH. More importantly, the data has augmented discussion of the utility of an integral biomarker as an enrichment strategy for optimum clinical trial candidate selection. The use of selected biomarkers appears to be linked with 13–21% relative increase in probability of successful transition between phases of clinical trial development [77].

Publication of all studies, irrespective of outcome, has the potential for cost reduction, improvement in future trial design, reduction in attrition rates and, most importantly, reduction in the number of clinical trial subjects exposed to ineffective drugs or enrolled in flawed clinical trial designs [83]. In fact, initiatives such as Medical Publishing Insights and Practices ([www.mpip-initiative.org](http://www.mpip-initiative.org)) and others supported by

pharmaceutical partners, the World Health Organization, and the Pharmaceutical Research and Manufacturers of America have called for transparency in publication of clinical trials protocols and results [83]. We in the PAH scientific community must recognise our ethical obligation to clinical trial subjects who allow us to expose them to investigational interventions with the hope that incremental knowledge will be gained. We are bound by this moral obligation to learn and disseminate knowledge produced by participation of each and every clinical trial subject. A transparent process for reporting and examining negative clinical trials, with the commitment to evaluation of full data sets (not only the top-line data) is essential. The process should exceed current data-sharing policies of individual institutions or medical journals and be funded to allow independently conducted analyses by scientific committees (*i.e.* data safety monitoring boards) with the necessary expertise (*i.e.* clinical trialist, ethicist and statistician). The obligation for such reporting processes should be balanced with (but not hindered by) the need for data confidentiality when analysing early-phase proof-of-concept studies.

### Challenges for future trial design

The successful development of drugs for PAH has provided significant impact on patient outcomes, but has not yet produced a cure. The surge of novel potential options has created new challenges in clinical care and future drug development programmes. Of course, this is a “good” stress to need to overcome, but it requires thoughtful consideration of novel trial end-points and design. It also means that pharmacological interactions, pharmacodynamics and individual pharmacokinetics will become more important to incorporate into future studies. Novel biomarkers and genetic association discovery will be required to truly develop and personalise therapies. There are still considerable unknowns regarding the mechanisms of action of our current drugs, their *in vivo* pharmacology and their optimal usage. Additionally, larger trials will, by necessity, need to be more collaborative in order to study potential meaningfulness of disease state biomarkers. Trials in PAH starting from the pre-clinical phase through phases 1 and 2 should focus on “learning” to improve efficiency of the entire enterprise. In addition, the number of patients with an orphan disease who are available to participate in clinical trials is limited. It is imperative to gain the most knowledge with the fewest patients placed at risk.

Future therapies can no longer be studied as *de novo* treatments with placebo-treated comparator groups. One solution to this dilemma is to implement creative adaptive designs. Informatics technology can allow real-time “adaptation” based on clinical data received, with adjustment of enrolment stratification and outcome targets, and determination of futility [84]. In addition, this technology can allow for testing multiple therapeutic choices if all stakeholders are willing to work together to find the best therapeutic effect. The 2018 US Food and Drug Administration guidance on adaptive designs for clinical trials defined these trials as “a study design that allows for prospectively planned modifications to one or more aspects of the design based on accumulating data from subjects in the trial” [85]. It is important to recognise that once interim monitoring occurs there is little statistical cost in having frequent monitoring from that point forward [86]. These Bayesian models allow statistical inferences to take into account the data-driven adaptations and thus allow trial flexibility [87].

The easiest trial to implement is a phase 2/3 trial that allows an interim “look” to assess whether the experimental arm is effective and therefore worth continuing on to its phase 3 stage [88]. This is a faster, more efficient way to proceed but is limited to trials utilising end-points in phase 2 that occur prior to reaching the definitive end-point needed for phase 3. Issues to address in advance include setting the response rate needed to proceed to phase 3, selecting the end-points and determining if there will be a time lag to allow for data to mature prior to analysis [89]. In contrast, a less traditional approach to be considered might be a multiarm trial with multiple experimental therapies tested against a single control arm [84]. This can be done with efficacy and futility stopping rules for each therapy, and potentially only dropping some of the arms while continuing the study with others [90].

Biomarker-driven trials typically consist of randomised block, marker-enriched and marker-directed designs [87]. Block design uses the biomarker as one of many factors used for randomisation, whereas the biomarker is central to the treatment assignment in marker-directed designs. Marker-enriched designs select a prognostic marker for treatment response or a determinant of higher risk to enhance success [87]. The main adaptive platform combining these concepts is the master protocol approach. The approach or “platform” allows adding new treatment arms in the existing cohort or in new subgroups to an ongoing trial in which several treatments are being tested at once [84]. The downside is that the later added arms can only be compared with the control patients randomised from the identical time-point of enrolment. This is not an easy task to organise as multiple industry partners need to collaborate in the trial design, control and privacy of data, funding, and regulatory requirements [91].

Use of biomarkers in adaptive design can strengthen trial efficiency [92]. Umbrella or basket trials describe master protocols designed to integrate proven molecular, genetic or serological biomarkers that are

associated with treatment response. This is now possible in oncology drug development and could be a potential design for PH trials. One example could be the use of a risk assessment tool as a biomarker at enrolment or during the study to stratify subjects. Eventually, the risk tools could then be modified to yield prognosis over time with these new markers. Biomarker-defined subgroups may be small proportions of the total cohort studied with this approach. In addition, the ability to discontinue the study of ineffective therapies and add an arm if the therapy is proven beneficial increases trial efficiency. Depending on the stage of the trial, the evidence required to meet a proven threshold will vary and will need consensus prior to trial initiation [84].

### Proposals for future clinical trial design in PAH

Collaboration and utilisation of existing resources are the keys to successful clinical development in PAH therapeutics. The ability to examine data upon completion in both successful and unsuccessful studies should be the rule, not the exception. Results of recent phase 1–2 studies with drugs targeting novel mechanisms of action have not yielded success, due to the heterogeneity of subjects studied, the use of myriad background therapies, the inclusion of patients with mild disease and perhaps selection of the wrong end-points. Rather than using traditional PAH clinical and haemodynamic trial end-points for targeted agents (e.g. 6MWT and PVR), the end-points should be tailored to the disease biology and anticipated mechanistic effects. This approach will allow for potential regulatory consideration of novel biomarkers.

#### Phase 1–2 goals

- 1) Pharmacokinetic and pharmacodynamics studies should be evaluated, particularly since we know little of the myriad combinations of therapies.
- 2) These trials should encompass biological and mechanistic markers of disease to better assess responses to therapy.
- 3) To improve generalisability and interpretation of the changes seen in functional capacity, early drug trials should include more homogenous subject groups.
- 4) Trials should use risk tools as an enrichment strategy to better phenotype a heterogeneous group.
- 5) Novel efficacy markers in phase 2 (e.g. magnetic resonance imaging and PROs) should be incorporated and then utilised in phase 3.

It is clear that performing long-term composite event-driven trials will be challenging and may halt the surge of new development by committing study participants for many years [93]. Morbidity and mortality event trials require large numbers of subjects upfront to demonstrate effects even at 1 year [35, 94]. Future clinical trials in PAH will need clinically meaningful end-points that reflect morbidity and mortality as well as how patients feel and function [95]. It is known that a “low-risk” patient profile after initial treatment of PAH is associated with better survival [96–99]: a potential exploratory end-point could be the ability to reach or maintain a “low-risk” status according to the REVEAL (Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management) score [96] or the number of low-risk criteria as defined by the European Society of Cardiology/European Respiratory Society PH guidelines [94, 98, 100, 101].

#### Phase 2–3 goals

The overarching goal should be to improve patients’ ability to live more functional and fulfilled lives.

- 1) Examine time to clinical *improvement* instead of TTCW. This will entail defining clinically significant differences in existing end-points and allowing for derivation of these differences post-trial with novel markers.
- 2) Utilise change in risk as a marker of efficacy as an exploratory end-point now to examine its predictability as a clinical end-point in the future.
- 3) Utilise novel biomarkers (serum, plasma, genomics, metabolics and PROs) as an exploratory end-point now to examine its predictability as a clinical end-point in the future.
- 4) Limit unnecessary post-marketing and small investigator-initiated studies that are unlikely to enhance our scientific knowledge base or improve patient care.

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