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Themed Issue: Pulmonary hypertension and right heart failure: new therapeutic avenue/approaches
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REVIEW ARTICLE

# Current and future treatments of pulmonary arterial hypertension

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Therapeutic options for pulmonary arterial hypertension (PAH) have increased over the last decades. The advent of pharmacological therapies targeting the prostacyclin, endothelin, and NO pathways has significantly improved outcomes. However, for the vast majority of patients, PAH remains a life-limiting illness with no prospect of cure. PAH is characterised by pulmonary vascular remodelling. Current research focusses on targeting the underlying pathways of aberrant proliferation, migration, and apoptosis. Despite success in preclinical models, using a plethora of novel approaches targeting cellular GPCRs, ion channels, metabolism, epigenetics, growth factor receptors, transcription factors, and inflammation, successful transfer to human disease with positive outcomes in clinical trials is limited. This review provides an overview of novel targets addressed by clinical trials and gives an outlook on novel preclinical perspectives in PAH.

**LINKED ARTICLES:** This article is part of a themed issue on Risk factors, comorbidities, and comedications in cardioprotection. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v178.1/issuetoc

**Abbreviations:** 6MWD, 6-min walk distance; ALK1, activin receptor-like kinase 1; ANP, atrial natriuretic peptide; ASK1, apoptosis signal-regulating kinase 1, MAP3K5; Bcl-2, B-cell lymphoma 2; BMP, bone morphogenetic protein; BMPR2, bone morphogenetic protein receptor 2; BNP, brain natriuretic peptide; BRD4, bromodomain-containing protein 4; CO, cardiac output; CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; DCA, dichloroacetate; DHEA, dehydroepiandrosterone; DMT, DNA methyltransferase; E2, oestradiol; ERAs, endothelin receptor antagonists; FDA, U.S. Food and Drug Administration; FHIT, fragile histidine triad; FKBP12, FK506-binding protein; FOX, forkhead box protein; HDACs, histone deacetylases; HIF, hypoxia-inducible factor; HMGB1, high mobility group box-1; IPAH, idiopathic pulmonary arterial hypertension; KCNK3, potassium channel subfamily K member 3 gene; mPAP, mean pulmonary arterial pressure; mTOR, mechanistic target of rapamycin; NAD+, nicotinamide adenine dinucleotide; NFAT, nuclear factor of activated T-cells; Nrf2, nuclear factor erythroid 2-related factor 2; p21, cyclin-dependent kinase inhibitor 1; p27, cyclin-dependent kinase inhibitor 1B; PA, pulmonary artery; PAEC, pulmonary arterial endothelial cells; PAH, pulmonary arterial hypertension; PASMCs, pulmonary arterial smooth muscle cells; PAWP, pulmonary arterial wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RCT, randomised controlled trial; ROCK, Rho-associated protein kinase; RTK, receptor TK; RUNX2, runt-related transcription factor 2; RV, right ventricle; SIRT, Sirtuin; SMURF-1, Smad ubiquitin regulatory factor 1; STAT, signal transducer and activator of transcription; TGFβR, TGFβ receptor; TPH1, tryptophan hydroxylase 1; UCP2, uncoupling protein 2; VCAM1, vascular cell adhesion molecule 1; VE/$VCO_2$, minute ventilation/carbon dioxide production; VIP, vasoactive intestinal peptide; $VO_2$max, maximal oxygen uptake.

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## 1 | DEFINITION AND CLASSIFICATION OF PULMONARY HYPERTENSION

Pulmonary hypertension (PH) was defined until recently by an elevated mean pulmonary arterial pressure (mPAP) of ≥25 mmHg (Galie et al., 2015). However, as the mPAP in healthy subjects was determined to be 14.0 ± 3.3 mmHg (Kovacs, Berghold, Scheidl, & Olschewski, 2009), the upper limit of normal, defined as two SDs above the mean value, mPAP should not exceed 20 mmHg (Simonneau et al., 2019). In the past, the relevance of the arbitrary definition of PH with a mPAP ≥25 mmHg has been questioned. Values >20 mmHg have clinical impact and affect outcome in populations at risk for PH (e.g., patients with systemic sclerosis; Simonneau et al., 2019; Valerio, Schreiber, Handler, Denton, & Coghlan, 2013). Thus, new cut-off values to define PH were suggested at the 6th World Symposium on PH in Nice 2018 defining PH as a mPAP >20-mmHg concomitant with a pulmonary vascular resistance (PVR) ≥ 3 Wood Units (WU) for pre-capillary forms of PH to account for the effect of cardiac output (CO) and pulmonary arterial wedge pressure (PAWP) on mPAP [PVR = (mPAP-PAWP)/CO] (Simonneau et al., 2019). It is currently unknown whether patients with mPAP between 21 and 24 mmHg and PVR ≥3 Wood Units would benefit from vasoactive treatment.

PH is currently classified into five separate groups with distinct pathophysiological characteristics (Galie et al., 2015). The clinical classification underwent minor revision at the 6th World Symposium (see Table 1; Simonneau et al., 2019). It ranges from rare forms such as pulmonary arterial hypertension (PAH, Group 1) and PH due to pulmonary artery obstructions (Group 4, primarily chronic thromboembolic PH [CTEPH]), to more common usually mild elevations of pressure seen in significant cardiac (PH due to left heart disease, Group 2) and respiratory disease (PH due to lung diseases and/or hypoxia, Group 3) and PH with unclear and/or multifactorial mechanisms (Group 5). The present review article will focus exclusively on PAH.

PAH has multiple causes but symptoms are similar in all forms of PAH, although their evolution and pattern vary depending on the aetiology. The most common symptoms are progressive breathlessness, fatigue, syncope, and clinical signs of heart failure. The diagnosis is usually first suggested by echocardiography. Confirmation of PAH requires right heart catheterisation and a systematic approach to investigation (Galie et al., 2015) including extensive imaging (Kiely et al., 2019) and integration with clinical features, to enable accurate classification which defines treatment. It is anticipated that advances in genotyping, improved imaging, and the application of artificial intelligence approaches to analysing data sets will aid refinement of disease classification and aid the study of treatment interventions. An overview of important landmarks in PAH research is given in Figure 1.

## 2 | OVERVIEW OF PATHOGENETIC MECHANISMS IN PAH

Pathological alterations have been recently reviewed in detail (Humbert et al., 2019). The initial concept that PAH is largely caused by mechanisms of vasoconstriction has been expanded over the last decades to a more complex picture in which multiple genetic, epigenetic, and environmental mechanisms lead to pulmonary vascular remodelling (Humbert et al., 2019). In some regards, PAH may even be considered as a pseudo-malignant disease with similar features to cancer (apoptotic resistance, altered metabolism, and overexpression of growth factor receptors; Boucherat, Vitry, et al., 2017). It is now accepted that curative therapeutic approaches must address not only vasoconstriction but also vascular remodelling, by inhibiting proliferative and activating anti-proliferative mechanisms (reverse remodelling; El Kasmi et al., 2014).

Pulmonary vascular remodelling underlying PAH is characterised by medial hypertrophy/hyperplasia, intimal and adventitial fibrosis, (in situ) thrombotic lesions, and plexiform lesions, as well as perivascular infiltration of inflammatory cells (B- and T-lymphocytes, mast cells, dendritic cells, macrophages, etc.; Humbert et al., 2019). It affects mainly distal muscular-type pulmonary arterial vessels and small pre-capillary arterioles (with diameters of 70–500 μm and 20–70 μm, respectively, in humans), but also to a varying degree post-capillary veins and bronchial arteries. The mechanisms for the latter are incompletely understood but may be connected by bronchial arterio-venous shunting (Humbert et al., 2019).

There is evidence demonstrating that all cell types of the vascular wall (fibroblasts, pulmonary arterial endothelial cells [PAEC], pulmonary arterial smooth muscle cells [PASMC], myofibroblasts, and pericytes) contribute to pulmonary vascular remodelling (Humbert et al., 2019). Several triggering factors combined with genetic/epigenetic susceptibility can initiate a phenotypical change of PAEC and PASMC characterised by apoptosis resistance, increased proliferation, and migration (Humbert et al., 2019). Damage of PAEC may play a particular role in the initiation of this process. Despite overabundance and/or overactivation of several pro-angiogenic

**TABLE 1** Clinical classification of PH and haemodynamic definitions (World Symposium on PH, Nice 2018)

| Classification | Haemodynamics[a] | Therapy |
| --- | --- | --- |
| Group 1: PAH<br>1.1 Idiopathic PAH<br>1.2 Heritable PAH<br>1.2.1 BMPR2 mutations<br>1.2.1 other mutations (ALK1, endoglin [with or without hereditary haemorrhagic telangiectasia])<br>1.3 Drug- and toxin-induced PAH<br>1.4 PAH associated (APAH) with<br>1.4.1 Connective tissue disease<br>1.4.2 HIV infection<br>1.4.3 Portal hypertension<br>1.4.4 Congenital heart disease<br>1.4.5 Schistosomiasis<br>1.5 PAH long-term responders to calcium channel blockers<br>1.6 PAH with overt features of venous/capillaries involvement (pulmonary veno-occlusive disease/pulmonary capillary haemangiomatosis)<br>1.7 Persistent PH of the newborn syndrome | Pre-capillary<br>mPAP > 20 mmHg<br>PAWP ≤ 15 mmHg<br>PVR ≥ 3 WU | Specific pulmonary vasoactive drugs<br>Supportive therapy<br>APAH: therapy of underlying disease |
| Group 2: PH due to left heart disease<br>2.1 PH due to heart failure with preserved LVEF<br>2.2 PH due to heart failure with reduced LVEF<br>2.3 Valvular heart disease<br>2.4 Congenital/acquired cardiovascular conditions leading to post-capillary PH | Isolated post-capillary<br>mPAP > 20 mmHg<br>PAWP > 15 mmHg<br>Combined pre-/post-capillary<br>mPAP > 20 mmHg<br>PAWP > 15 mmHg<br>PVR ≥ 3 WU | Therapy of underlying disease |
| Group 3: PH due to lung diseases and/or hypoxia<br>3.1 Obstructive lung disease<br>3.2 Restrictive lung disease<br>3.3 Other lung disease with mixed restrictive/obstructive pattern<br>3.4 Hypoxia without lung disease<br>3.5 Developmental lung disorders | Pre-capillary<br>mPAP > 20 mmHg<br>PAWP ≤ 15 mmHg<br>PVR ≥ 3 WU<br>Severe PH:<br>mPAP ≥ 35 mmHg | Therapy of underlying disease<br>Oxygen supplementation |
| Group 4: PH due to pulmonary artery obstructions<br>4.1 Chronic thromboembolic PH<br>4.2 Other pulmonary artery obstructions<br>4.2.1 Sarcoma (high or intermediate grade) or angiosarcoma<br>4.2.2 Other malignant tumours<br>4.2.3 Non-malignant tumours<br>4.2.4 Arteritis without connective tissue disease<br>4.2.5 Congenital pulmonary artery stenoses<br>4.2.6 Parasites: Hydatidosis | Pre-capillary<br>mPAP > 20 mmHg<br>PAWP ≤ 15 mmHg<br>PVR ≥ 3 WU | Anticoagulation[b]<br>Pulmonary endarterectomy[b]<br>Balloon pulmonary angioplasty[b]<br>Specific pulmonary vasoactive drugs[b] |
| Group 5: PH with unclear and/or multifactorial mechanisms<br>5.1 Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders<br>5.2 Systemic and metabolic disorders: pulmonary Langerhans cell | Pre-capillary<br>mPAP > 20 mmHg<br>PAWP ≤ 15 mmHg<br>PVR ≥ 3 WU<br>Isolated post-capillary<br>mPAP > 20 mmHg | Therapy of underlying disease |

(Continues)

**TABLE 1** (Continued)

| Classification | Haemodynamics[a] | Therapy |
|---|---|---|
| histiocytosis, Gaucher disease, glycogen storage disease, neurofibromatosis, sarcoidosis | PAWP > 15 mmHg<br>PVR < 3 WU | |
| 5.3 Others: chronic renal failure with or without haemodialysis, fibrosing mediastinitis | Combined pre-/post-capillary<br>mPAP > 20 mmHg<br>PAWP > 15 mmHg | |
| 5.4 Complex congenital heart disease | PVR ≥ 3 WU | |

*Note.* Grey shading means main post-capillary form of PH.

Abbreviations: ALK, activin receptor-like kinase; APAH, associated pulmonary arterial hypertension; BMPR, bone morphogenic protein receptor; HIV, human immunodeficiency virus; LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension.

[a]According to the 6th World Symposium on PH (Simonneau et al., 2019).

[b]Treatment of chronic thromboembolic PH requires a multifaceted approach which may include multiple interventions.

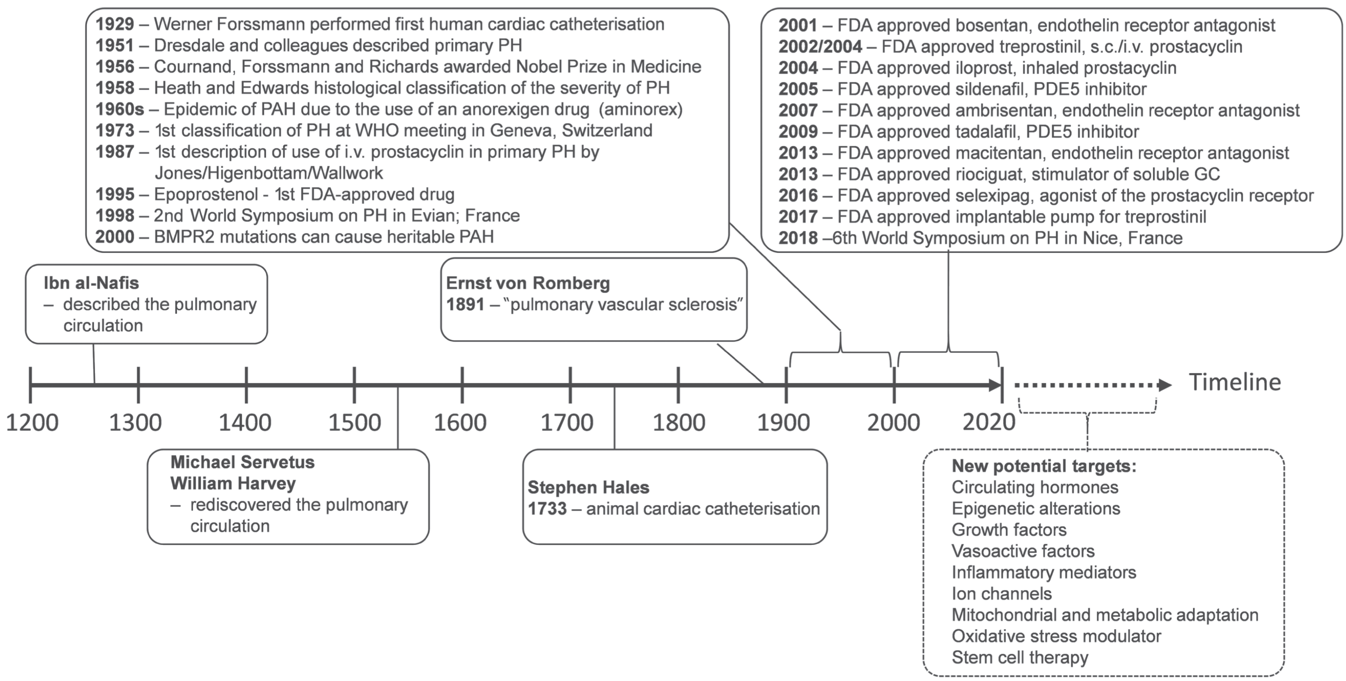


**FIGURE 1** History of PH and development of therapeutic drugs. FDA, U.S. Food and Drug Administration; WHO, World Health Organisation

pathways, angiogenesis and pericyte-associated tube formation of endothelial cells is disturbed leading to progressive obliteration of pre-capillary arteries ("vascular pruning" or "dead-tree" picture; Humbert et al., 2019; Yuan et al., 2015). Furthermore, fibroblasts become hyperproliferative and are involved in remodelling of the extracellular matrix promoting the alterations of PASMC and PAEC, as well as increasing stiffness of the large elastic main, lobar, and segmental pulmonary arteries (Humbert et al., 2019).

PAH is a multifactorial and heterogeneous disease in which multiple and different pathogenetic alterations have been observed in similar phenotypes (Figure 2): (1) Environmental trigger factors include air pollution (Sofianopoulou et al., 2019), as well as (particularly for PH Group 3) hypoxia and smoke exposure, shear stress (PH Group 1.4.4) and infections (PH Groups 1.4.2 and 1.4.5). (2) Different mutations leading to genetic susceptibility (bone morphogenetic protein receptor 2 [BMPR2], activin receptor-like kinase 1 [ALK1], voltage-gated potassium channel 1.5 [$K_v$1.5], and potassium channel subfamily K member 3 [KCNK3]) have been identified, and early life epigenetic imprinting may also play a role. (3) PAH is promoted or triggered by systemic and circulating factors such as hormones and metabolites, as well as a pro-coagulatory and inflammatory disposition, particularly in PH Group 1.4.1. (4) Endothelial dysfunction leads to an imbalanced release of endothelial factors (endothelin, Tx, NO, and prostacyclin) and is currently targeted by PAH therapy. Further endothelial mechanisms include reduced anticoagulatory endothelial properties, increased expression of adhesion molecules (E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecules), and endothelial release of different chemokines, cytokines, and growth factors (Huertas et al., 2018). (5) Cellular mechanisms in PAEC, PASMC, and fibroblasts include altered expression/function of ion channels and growth factor receptors, activation or deactivation of transcription factors (e.g., nuclear factor of

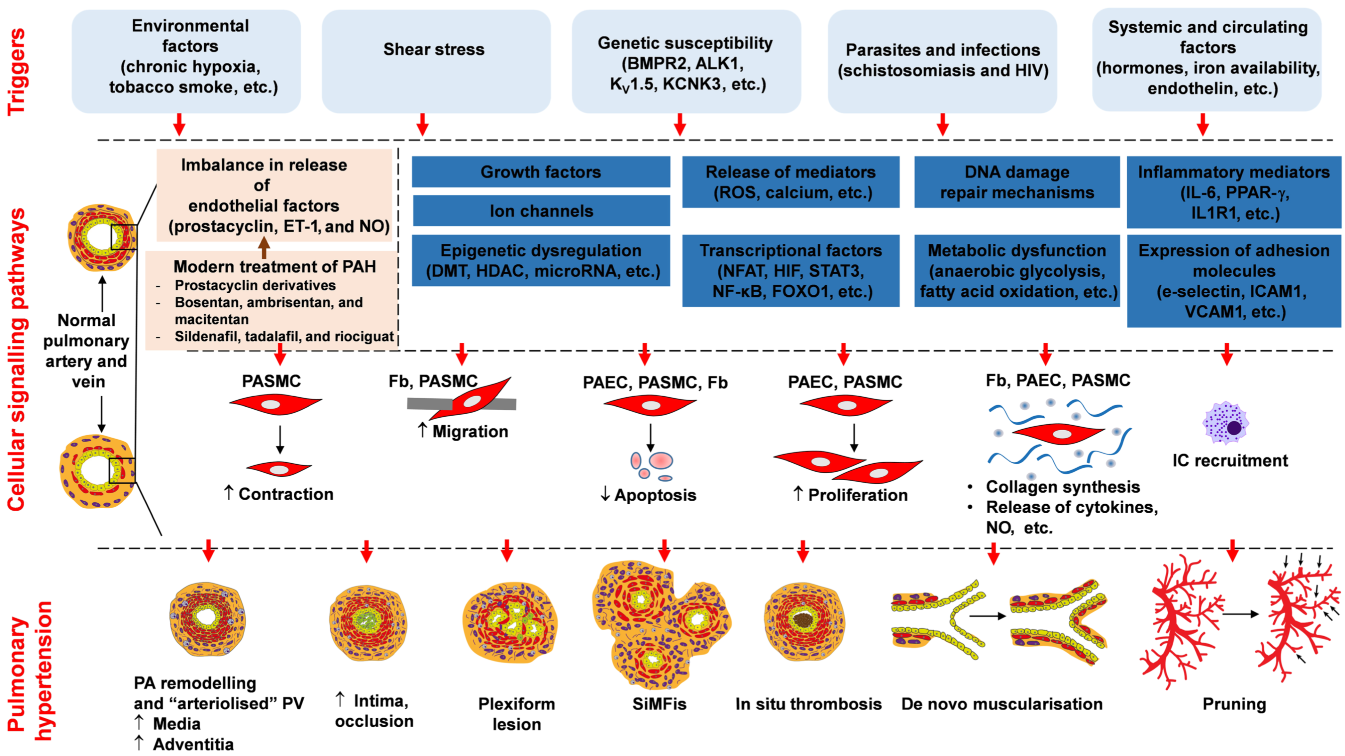


**FIGURE 2** Patho-mechanisms underlying PAH. For details, please refer to text. DMT, DNA methyltransferase; ET, endothelin; Fb, fibroblasts; IC, immune cell; IL1R1, IL-1 receptor type 1; PA, pulmonary artery; PV, pulmonary veins; SiMFis, singular millimetric fibrovascular lesions

activated T-cells [NFAT], hypoxia-inducible factor [HIF] 1, signal transducer and activator of transcription [STAT] 3, and forkhead box protein [FOX] O1), and dysregulated cellular metabolism. (6) Finally, repair mechanisms counteracting remodelling are disturbed, including DNA and endothelial repair mechanisms (Boucherat, Vitry, et al., 2017; Humbert et al., 2019).

The multifactorial nature of PAH may be one of the reasons why finding a cure for PAH is challenging. For example, despite the importance of BMPR2 in the pathobiology of PAH, disease penetrance in carriers is only ~20% (higher in females than males), indicating that other factors are required for development of the phenotype. There are currently studies ongoing in large populations with idiopathic PAH (IPAH) and heritable PAH to identify further mutations or polymorphisms that could represent a "second hit." Several candidates have been identified, including variants in the prostacyclin synthase gene and mutations in the potassium voltage-gated channel sub-family A member 5 gene (Ghataorhe et al., 2017).

## 3 | CURRENT TREATMENT OPTIONS IN PAH

Treatment of PAH currently includes supportive therapy such as diuretics, supervised rehabilitation, birth control advice, and oxygen supplementation, if needed (Ulrich et al., 2019). Pharmacological therapies targeting the pulmonary vasculature are currently only approved for patients with PAH (Group 1) and selected patients with CTEPH (Group 4). In other forms of PH, clinical trials using therapies approved for PAH have yet to demonstrate benefit, and current treatment strategies aim to optimise therapy of the underlying disease (see Table 1).

In PAH, current pharmacological therapies target three pathways regulating endothelial factors with vasoconstrictive/vasodilatory and proliferative/mitogenic properties: (1) NO-cGMP signalling is targeted by PDE5 inhibitors (tadalafil and sildenafil) and a soluble GC stimulator (riociguat); (2) endothelin receptor antagonists (ERAs) target both $ET_A$ and $ET_B$ receptors non-selectively (bosentan) or $ET_A$ receptors selectively (ambrisentan and macitentan. This selectivity may be advantageous because stimulation of $ET_A$ receptors causes vasoconstriction whereas stimulation of $ET_B$ receptors causes vasodilation); and (3) prostacyclin signalling is increased by either parenteral prostacyclin analogues (epoprostenol, treprostinil, and iloprost) or an orally available IP receptor agonist (selexipag; Galie et al., 2015). Milestones in the development of PAH therapies are shown in Figure 1, and pivotal studies are summarised in Table 2. In addition, a small group of patients with idiopathic, heritable, or drug-induced PAH who respond to a vasodilator challenge with a significant drop in pulmonary artery pressures and who have a sustained response to high-dose calcium channel blockade (around 5–10% of patients with IPAH) have excellent long-term survival (Rich, Kaufmann, & Levy, 1992; Sitbon

**TABLE 2** Clinical trials in PAH

| Trial name and identification number | Type of trial | Primary endpoint | Reference or trial duration/start date and status |
|---|---|---|---|
| A. Landmark clinical trials in PAH positive for primary endpoint and resulting in approval of therapy | | | |
| Continuous infusion of i.v. epoprostenol | RCT: 81 patients with IPAH | Change in 6MWD and PVR | Barst R.J., *N Engl J Med* 1996; 334: 296–301 |
| Continuous s.c. infusion of treprostinil | RCT: 470 patients with PAH | Change from baseline in 6MWD at 12 weeks | Simonneau G. et al., *Am J Respir Crit Care Med* 2002; 165: 800–804 |
| Aerosolised iloprost (AIR) | RCT: 203 treatment-naïve patients with mixed types of PH | An increase of at least 10% in 6MWD and 1 NYHA FC at 12 weeks | Olschewski H. et al., *N Engl J Med* 2002; 347: 322–329 |
| Bosentan (BREATHE-1) | RCT: 213 treatment-naïve patients with either IPAH or CTD-PAH | Change from baseline in 6MWD at 16 weeks | Rubin L.J. et al. *N Engl J Med* 2002; 346: 896–903 |
| Sildenafil (SUPER) | RCT: 278 treatment-naïve patients with PAH | Change from baseline in 6MWD at Week 12 | Galiè N. et al., *N Engl J Med* 2005; 353: 2148–2157 |
| Ambrisentan (ARIES-1 and ARIES-2) | RCT: 202 (ARIES-1) and 192 (ARIES-2) treatment-naïve patients with PAH | Change from baseline in 6MWD at Week 12 | Galiè N. et al., *Circulation* 2008; 117: 3010–3019 |
| Tadalafil (PHIRST-1) | RCT: 405 treatment-naïve or pretreated patients with PAH | Change from baseline in 6MWD at Week 16. Endpoint not met in pretreated patients | Galiè N. et al., *Circulation* 2009; 119: 2894–2903 |
| Inhaled treprostinil (TRIUMPH I) | RCT: 235 pretreated patients with PAH | Change from baseline in 6MWD at 12 weeks | McLaughlin V. et al., *J Am Coll Cardiol* 2010; 55: 1915–1922 |
| Macitentan (SERAPHIN) | RCT: 742 treatment-naïve or pretreated patients with PAH | Time from initiation of treatment to first occurrence of a composite morbidity or mortality endpoint | Pulido T. et al., *N Engl J Med* 2013; 369: 809–818 |
| Riociguat (PATENT-1/2) | RCT: 443 treatment-naïve or pretreated patients with PAH | Change from baseline in 6MWD at Week 12 | Ghofrani H.A. et al. *N Engl J Med* 2013; 369: 330–340 |
| Selexipag (GRIPHON) | RCT: 1156 treatment naïve or pretreated patients with PAH | Time from initiation of treatment to first occurrence of a composite morbidity or mortality endpoint | Sitbon O. et al *N Engl J Med* 2015; 373: 2522–2533 |
| Clinical trials of up-front combination therapy in PAH positive for primary endpoint | | | |
| Ambrisentan and tadalafil in combination versus montherapy (AMBITION) | RCT: 500 treatment naïve patients with PAH | Time from initiation of treatment to first occurrence of a composite morbidity or mortality endpoint | Galie N. et al., *N Engl J Med* 2015; 373: 834–844 |
| B. Trials in PAH not positive for primary endpoint, terminated early or other results | | | |
| Terguride: 5-HT receptor antagonist | Phase 2 trial: 78 patients with PAH | Change from baseline in PVR at Week 16 | Ghofrani et al., 2012 (abstract)<br>Endpoint not reached |
| Nilotinib: TK inhibitor (AMN107) NCT01179737 | Phase 2 trial: 23 patients with PAH | Change from baseline in PVR at 6 months | 2010–2014<br>Terminated due to serious adverse event |
| Aspirin and simvastatin: COX and HMG-CoA-reductase inhibitor | Phase 2 trial: 92 patients with PAH | Change from baseline in 6MWD at 6 months | Kawut S.M. et al., *Circulation* 2011; 123 (25): 2985–2993<br>Endpoint not reached |
| Atorvastatin: HMG-CoA-reductase inhibitor (APATH) | Phase 2 trial: 220 patients with PAH and CTEPH | Change from baseline in 6MWD at 24 weeks | Zeng W.J. et al., 2012; 40 (1): 67–74<br>Endpoint not reached |
| Inhaled aviptadil: VIP | Phase 2 trial: 56 patients with PAH | Change in PVR acute or after 3 months | Published as abstract: Galiè et al., *Am J Respir Crit Care Med* 2010; 181: A2516 |

(Continues)

**TABLE 2** (Continued)

| Trial name and identification number | Type of trial | Primary endpoint | Reference or trial duration/start date and status |
|---|---|---|---|
| | | | Said S.I., *Am J Respir Crit Care Med* 2012 Apr 1; 185 (7): 786; author reply 786<br>Endpoint not reached |
| Imatinib: TK inhibitor (QTI571; IMPRES) | Phase 3 trial: 202 patients with PAH | Change from baseline in 6MWD at Week 24 | Hoeper M.M. et al., *Circulation* 2013; 127 (10): 1128–1138. Endpoint reached but safety concerns |
| Selonsertib: ASK1 inhibitor (ARROW) | Phase 2 trial: 150 patients with PAH | Change from baseline in PVR at Week 24 | Published as abstract: Boucherat O. et al., *Am J Respir Crit Care Med* 197 (3): 284–286, 2018<br>Endpoint not reached |
| FK506: calcineurin inhibitor (tacrolimus) | Phase 2 trial: 23 patients with PAH | Frequency of adverse events during 16 weeks | Spiekerkoetter E. et al., *Eur Respir J.* 2017; 50 (3)<br>Low-level FK506 is well tolerated and increases BMPR2 in subsets of patients with PAH |
| Pioglitazone: dipeptidyl peptidase 4 inhibitor NCT00825266 | Phase 2 trial: 2 patients with PAH | Insulin resistance profile change at Week 16 | 2009–2017<br>Terminated (difficulty in finding eligible patients) |
| Ubenimex: aminopeptidase inhibitor (LIBERTY1/2) NCT02664558/ NCT02736149 | Phase 2 trial: 61/51 patients with PAH | Change from baseline in PVR at Week 24/frequency of adverse events | 2016–2018<br>Terminated due to failure of efficacy |
| Racecadotril: neprilysin inhibitor | Phase 2 trial: 21 patients with PAH | Maximum change in circulating ANP concentration after 14 days | Hobbs A.J. et al. *Br J Pharmacol* 2019 May; 176 (9): 1251–1267<br>Increase in ANP |
| Anakinra: IL-1 inhibitor | Phase 1 trial: 7 patients with PAH | $VO_2$max, VE/$VCO_2$ slope at 14 days | Trankle C.R. et al., *Am J Respir Crit Care Med* 2019; 199 (3): 381–384<br>Endpoint not reached |
| Ambrisentan plus spironolactone: ERA plus aldosterone antagonist NCT02253394 | Phase 4 trial: 30 patients with PAH | Cardiopulmonary fitness at 200 days | 2014–2019<br>Terminated (low enrolment) |
| Fulvestrant: oestrogen antagonist NCT02911844 | Phase 2 trial: 5 patients with PAH | Change from baseline in oestradiol, tricuspid annular plane systolic excursion, 6MWD, and N-terminal pro-brain natriuretic peptide at 9 weeks | Kawut S.M. et al., *Ann Am Thorac Soc.* 16 (11): 1456–1459, 2019<br>No significant changes of endpoints |
| Tocilizumab: anti-IL-6 antibody (TRANSFORM-UK) NCT02676947 | Phase 2 trial: 29 patients with PAH | Adverse events; change from baseline in PVR at 6 months | 2016–2018<br>Completed, results posted online<br>Endpoint not reached |
| C. Current ongoing clinical trials on PAH | | | |
| Clinical trials of up-front combination therapy in PAH using licensed therapies | | | |
| Macitentan and tadalafil and selexipag versus macitentan and tadalafil in combination (TRITON) NCT02558231 | RCT: 238 treatment naïve patients with PAH | Change from baseline in PVR at Week 26 | 2016<br>Recruiting |
| Hormonal modulators | | | |
| Anastrozole: aromatase inhibitor (PHANTOM) NCT03229499 | Phase 2 trial: 84 patients with PAH | Change from baseline in 6MWD at 6 months | 2017<br>Recruiting |
| | Phase 2 trial: 24 patients with PAH | | 2018<br>Recruiting |

(Continues)

**TABLE 2** (Continued)

| Trial name and identification number | Type of trial | Primary endpoint | Reference or trial duration/start date and status |
|---|---|---|---|
| Tamoxifen: oestrogen receptor inhibitor (T3PAH) NCT03528902 | | Change from baseline in tricuspid annular plane systolic excursion at Week 24 | |
| DHEA (EDIPHY) NCT03648385 | Phase 2 trial: 24 patients with PH | Change in RV longitudinal strain between DHEA and placebo at Weeks 18 and 40 | 2018 Recruiting |
| Spironolactone: aldosterone antagonist NCT01712620 | Phase 2 trial: 70 patients with PAH | Change from baseline in 6MWD at 6 months | 2012 Recruiting |
| rhACE2: GSK2586881 NCT03177603 | Phase 2 trial: 24 patients with PAH | Change from baseline in PVR up to 4 hr | 2017 Recruiting |
| KAR5585: tryptophan hydroxylase 1 inhibitor NCT02746237 | Phase 1 trial: 120 healthy individuals | Adverse events up to 4 days | 2016–2016 Completed, results pending |
| Escitalopram: SSRI NCT00190333 | Phase 3 trial: 30 patients with PH | Change from baseline in 6MWD at Week 16 | 2005–2008 Completed, results pending |
| Fluoxetine: SSRI NCT03638908 | Phase 2 trial: 8 patients with WHO group 1 PH | Change from baseline in PVR at Week 24 | 2018 Active, not recruiting |
| PB1046: VIP analogue NCT03315507 | Phase 1 trial: 10 patients with WHO group 1 PH | Adverse events 28 days after last dose, laboratory parameters, change in PVR | 2017 Active, not recruiting |
| GPCR pathways | | | |
| Apelin (EXAP) NCT01590108 | Phase 1 trial: 12 healthy individuals and patients with IPAH | Cardiopulmonary performance at 6 months | 2012–2019 Completed, results pending |
| Mitochondrial and metabolic adaptations | | | |
| Ranolazine: sodium channel inhibitor, partial FAO inhibitor NCT01839110 | Phase 4 trial: 22 patients with PAH | Change in RVEF by MRI at 6 months | 2013–2019 Completed, results pending |
| Ranolazine NCT02829034 | Phase 4 trial: 22 patients with PAH | Change from baseline in RVEF by MRI at Week 26 | 2016–2019 Completed, results pending |
| Trimetazidine: FAO inhibitor NCT02102672 | Phase 2 trial: 25 patients with PAH | Changes in RV function assessed by 3D echo at 3 months | 2014 Recruitment state unknown |
| Metformin: biguanide NCT03617458 | Phase 2 trial: 160 patients with PAH | Change from baseline in 6MWD at Week 12 | 2018 Recruiting |
| Ferinject or CosmoFer: iron infusion NCT01447628 | Phase 2 trial: 40 patients with PAH | Change from baseline in PVR and endurance at Week 12 | 2011–2019 Completed, results pending |
| Epigenetic alterations and interaction with metabolic pathway | | | |
| Olaparib: PARP inhibitor (OPTION) NCT03782818 | Phase 1 trial: 20 patients with PAH | Adverse events at Week 24 | 2019 Not yet recruiting |
| Apabetalone: BRD4 *inhibitor* (APPRoAcH-p) NCT03655704 | Phase 1 trial: 10 patients with PAH | Change from baseline in PVR at Week 16 | 2019 Not yet recruiting |
| Oxidative stress related pathways | | | |
| Bardoxolone methyl: IκB kinase and NF-κB inhibitor, Nrf2 activator (LARIAT) NCT02036970 | Phase 2 trial: 166 patients with PH | Change from baseline in 6MWD at Week 16 | 2014–2019 Completed, results pending |
| Bardoxolone methyl (CATALYST) NCT02657356 | Phase 3 trial: 200 patients with CTD-PAH | Change from baseline in 6MWD at Week 24 | 2016 Recruiting |
| Bardoxolone methyl (RANGER) NCT03068130 | Phase 3 trial: 414 patients with PH | Long-term safety up to 5 years | 2017 Recruiting |
| CXA-10: nitrated fatty acid compound (PRIMEx) NCT03449524 | Phase 2 trial: 96 patients with PAH | Change in RVEF and PVR at 6 months | 2018 Recruiting |

(Continues)

**TABLE 2** (Continued)

| Trial name and identification number | Type of trial | Primary endpoint | Reference or trial duration/start date and status |
|---|---|---|---|
| Inflammatory mediators | | | |
| Rituximab: anti-CD20 antibody NCT01086540 | Phase 2 trial: 58 patients with systemic sclerosis associated-PAH | Change in PVR at Week 24 | 2010<br>Active, not recruiting |
| Elafin: elastase-specific protease inhibitor NCT03522935 | Phase 1 trial: 30 healthy individuals | Adverse events at 28 days | 2018<br>Recruiting |
| Growth factor receptors | | | |
| Sotatercept: activin receptor type 2A fusion protein acting as a ligand trap (SPECTRA) NCT03738150 | Phase 2 trial: 25 patients with PAH | Change from baseline in $VO_2$ max at Week 24 | 2018<br>Recruiting |
| Sotatercept (PULSAR) NCT03496207 | Phase 2 trial: 100 patients PAH | Change from baseline in PVR at Week 24 | 2018<br>Recruiting |
| Transcriptional factors | | | |
| ABI-009: mTOR inhibitor NCT02587325 | Phase 1 trial: 25 patients with PAH | Adverse events at Week 16 | 2015<br>Recruiting |
| Stem cell therapy | | | |
| Allogeneic cardiosphere-derived stem cells NCT03145298 | Phase 1 trial: 26 patients with PAH | Primary safety endpoints within 72 hr of infusion | 2017<br>Recruiting |
| eNOS-enhanced EPCs NCT03001414 | Phase 2 trial: 45 patients with PAH | Change from baseline in 6MWD at Month 6 | 2016<br>Recruiting |

Abbreviations: 6MWD, 6-min walk distance; ANP, atrial natriuretic peptide; ASK1, apoptosis signal-regulating kinase 1; BRD4, bromodomain-containing protein 4; CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension; DHEA, dehydro-epiandrosterone; eNOS, endothelial NOS; EPCs, endothelial progenitor cells; FAO, fatty acid oxidation; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; mTOR, mechanistic target of rapamycin; Nrf2, nuclear factor erythroid 2-related factor 2; NYHA FC, New York Heart Association functional classification; RCT, randomised controlled trial; rhACE2, recombinant human ACE2; RVEF, right ventricular ejection fraction; SSRI, selective serotonin reuptake inhibitor; $VE/VCO_2$, minute ventilation/carbon dioxide production; VIP, vasoactive intestinal peptide; $VO_2$max, maximal oxygen uptake.

et al., 2005). This serves to illustrate the heterogeneous nature of IPAH and the importance of developing a personalised approach to drug therapy.

Recent improvements in outcomes for patients with PAH reflect a move to the use of combination drug therapy targeting multiple pathways. An overview of clinical trials of combination therapy in PAH was recently provided (Humbert & Ghofrani, 2016). Current treatment algorithms are based on interval multiparameter risk assessment with the aim of achieving a low-risk status (Galie et al., 2019). Lung transplantation remains an important therapeutic option for patients deteriorating despite maximal medical therapy. Despite treatment advances and an increase in survival by more than twofold during the last two decades, PAH remains a fatal disease, and the identification of new targets and development of new therapies is required.

## 4 | NOVEL TREATMENT OPTIONS TARGETING SPECIFIC PATHWAYS IN PAH

Preclinical and clinical research on novel drugs currently focusses on PAH where typical characteristics of pulmonary vascular remodelling exist. This review describes novel concepts that have been established in preclinical models and have been transferred to clinical trials (Figure 3; ongoing clinical trials are summarised in Table 2). Furthermore, some very new studies on preclinical approaches are described. Although preclinical models only partly reflect features of PAH, they have been successfully used to test treatment options in PAH. The most common preclinical models are the chronic hypoxic model of the mouse and rat leading to rather mild PH without characteristic plexiform lesions, the combined application of hypoxia and the VEGF inhibitor Sugen 5416 (Sugen/hypoxia) in rats which is characterised by more severe PH including plexiform lesions, and the monocrotaline model of rats which is largely driven by inflammatory stimuli and causes severe PH (Stenmark, Meyrick, Galie, Mooi, & McMurtry, 2009). Several other models with knockdown of relevant pathways have been described, but none of the models reproduces all the histopathological patterns of PAH.

### 4.1 | Circulating hormones

#### 4.1.1 | Sex hormones

Systemic factors, mainly hormones or nutrients, may either trigger or promote the development of PAH. The best known hormone-

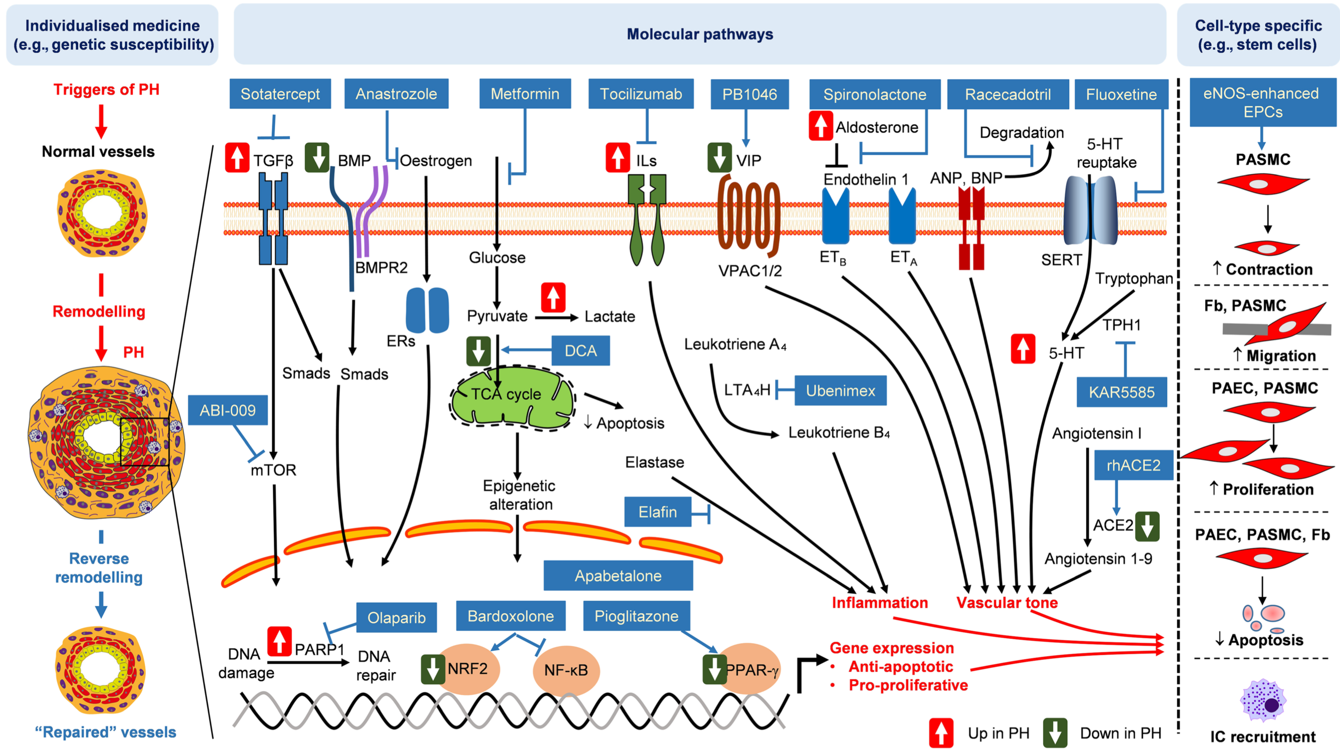


**FIGURE 3** Novel treatment options in PAH. Substances shown in the Figure are currently tested in clinical trials. For details, please refer to text. EPCs, endothelial progenitor cells; ERs, oestrogen receptors; $LTA_4H$, $LTA_4$ hydrolase; SERT, 5-HT (serotonin) transporter; VPAC, vasoactive intestinal peptide receptor

related phenomenon in this regard is the "oestrogen paradox": Women have a higher incidence of IPAH and heritable PAH than men, but female patients with PAH have better outcomes than male patients. The role of different oestradiol (E2) metabolites and receptors is not completely elucidated. Some of the metabolites have proliferative effects (e.g., 16α-hydroxyoestrone), while others also have anti-proliferative and anti-inflammatory properties (e.g., 2-hydroxyoestradiol and 2-methoxyoestradiol). High E2 levels were associated with PAH in male patients and reduced exercise capacity in female patients, but E2 may also have protective effects on the right ventricle (RV; recently reviewed in Tofovic & Jackson, 2019). Thus, different levels of E2 and its metabolites at different disease stages could explain sex-dependent differences. Moreover, genes of the Y-chromosome are up-regulated in PH and have protective anti-remodelling properties in hypoxic mice (Umar et al., 2018). Decreasing circulating E2 by inhibiting conversion of androgens to E2 using the aromatase inhibitor anastrozole and inhibition of the E2 receptor with tamoxifen inhibited PH in different animal models of PH, partly in a sex-dependent way (Tofovic & Jackson, 2019). By contrast, supplementation with E2 increased exercise capacity in male and female rats with severe PH, probably by exerting protective effects on the RV (Lahm et al., 2016). Most importantly, anastrozole showed good safety and tolerability in male and female patients with PAH in a small "proof-of-principle" trial and significantly reduced E2 levels and improved 6-min walk distance (6MWD) but not RV function (Kawut et al., 2017). These pilot data supported initiation of the randomised, controlled PH and Anastrozole Trial (PHANTOM; ClinicalTrials.gov identifier: NCT03229499). Furthermore, the effect of oestrogen inhibition with tamoxifen will be investigated in patients with PAH in a small randomised controlled trial (RCT; Tamoxifen Therapy to Treat PAH [T3PAH]; ClinicalTrials.gov identifier: NCT03528902). In contrast to anastrozole, tamoxifen can be used in pre-menopausal female patients without inducing menopause. An open-label trial of the oestrogen antagonist fulvestrant (ClinicalTrials.gov identifier: NCT02911844) was recently completed. However, due to the low number of participants, no final conclusions on the effects of fulvestrant on PAH can be drawn. Nevertheless, it was generally well tolerated (Kawut et al., 2019).

### 4.1.2 | Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is a steroid hormone that serves as a precursor for both oestrogen and testosterone synthesis. DHEA prevented and reversed PH and RV dysfunction in different animal models of PH (Lahm, Tuder, & Petrache, 2014). Accordingly, higher levels of E2 and lower levels of DHEA were associated with increased risk of PAH in men (Ventetuolo et al., 2016) and increased risk and severity of PAH in post-menopausal women (Baird et al., 2018). DHEA treatment significantly improved 6MWD, pulmonary haemodynamics, and diffusion capacity of patients with PH associated with chronic obstructive pulmonary disease, without worsening gas exchange (de La Roque et al., 2012). Currently, DHEA is being tested in a crossover trial in a small number of patients with PAH (Effects of DHEA in PH [EDIPHY]; ClinicalTrials.gov identifier: NCT03648385). The primary outcome is change in RV longitudinal strain, as determined by cardiac MRI.

#### 4.1.3 | Renin–angiotensin–aldosterone system

The concept of transferring therapeutic approaches from the systemic to the pulmonary circulation has refocussed attention on the role of the neurohumoral and renin–angiotensin–aldosterone systems (Maron & Leopold, 2015). Antagonists of the receptor for aldosterone, a steroid hormone that binds the **mineralocorticoid receptor** in the heart and pulmonary vasculature, have been used for fluid management in PAH. In animal studies, aldosterone antagonists attenuated or partially reversed PH (Preston et al., 2013). However, despite the potential of aldosterone antagonists to exert beneficial effects on the RV and pulmonary vasculature beyond their diuretic properties, no RCT has yet evaluated their efficacy in PAH. The "Combination Ambrisentan Plus Spironolactone in Pulmonary Arterial Hypertension Study" (CAPS-PAH)—a crossover study investigating whether addition of **spironolactone** to ambrisentan affects exercise capacity in patients with PAH—was terminated because of low enrolment (ClinicalTrials.gov identifier: NCT02253394). Another RCT will examine the effect of spironolactone on 6MWD and clinical worsening in patients with PAH (ClinicalTrials.gov identifier: NCT01712620).

Another novel approach addresses the peptidase **ACE2** which converts the peptide hormones **angiotensin I** and **angiotensin II** to their vasodilator derivates (**angiotensin-(1–9)** and **angiotensin-(1–7)**, respectively) with a preference for angiotensin II degradation. However, ACE2 may also degrade other vasodilatory factors such as **apelin** (see below; Kazemi-Bajestani, Patel, Wang, & Oudit, 2012). Decreased ACE2 levels and ACE2 autoantibodies have been reported in PAH (Tan, Liao, Zhou, Mei, & Wong, 2018). An open-label pilot study on acute haemodynamic responses after a single infusion of recombinant human ACE2 in patients with PAH showed improved CO without a significant change in mPAP or systemic pressures, and reduced markers of oxidant and inflammatory stress (Hemnes et al., 2018). A dose-escalation study in PAH is currently recruiting patients (ClinicalTrials.gov identifier: NCT03177603).

#### 4.1.4 | Atrial natriuretic peptide

**Atrial natriuretic peptide (ANP)** and **brain natriuretic peptide (BNP)** are released from atrial cardiomyocytes or the ventricles respectively. Natriuretic peptides increase renal sodium excretion, cause vasodilation via cGMP release, and decrease heart fibrosis. Natriuretic peptides are degraded by the metalloprotease **neprilysin**. Recently, a combination of the angiotensin receptor blocker **valsartan** and the neprilysin inhibitor **sacubitril** was approved for treatment of left heart failure (McMurray et al., 2014). The combination treatment was used because neprilysin degrades not only natriuretic peptides but also vasoconstrictive factors such as endothelin and angiotensin II. Studies of neprilysin in animal models of PH showed conflicting results. While neprilysin-deficient mice exhibited an exaggerated response to chronic hypoxia (Dempsey et al., 2009), pharmacological inhibition of neprilysin alone or in combination with **PDE5** inhibition attenuated PH in chronic hypoxic rats without affecting systemic circulation (Baliga et al., 2008; Thompson, Sheedy, & Morice, 1994). Recently, the efficacy and safety of the neprilysin inhibitor racecadotril was tested in an RCT in PAH. Acute administration increased plasma ANP and cGMP levels and slightly decreased PVR. Similar to the ACE2 trial, mPAP did not change significantly, indicating that the decrease in PVR was mostly driven by an increase in CO. Systemic BP was not decreased significantly, and plasma endothelin levels did not change following exposure to racecadotril (Hobbs et al., 2019). Further studies are necessary to investigate the effect of these vasodilators on PAH.

#### 4.1.5 | Neurohormonal regulation

Neurohormonal regulation plays an important role in pulmonary vascular tone and RV function (Maron & Leopold, 2015). In fact, β-blockers (β-adrenoceptor antagonists) are one of the main treatment strategies in left ventricular systolic dysfunction and may thus have a place in PAH therapy (beyond their indication for supraventricular tachycardia which is a frequent co-morbidity in PAH). Animal studies suggest that β-blockers may have beneficial effects on RV dysfunction and maladaptive remodelling. It has also been suggested that some β-blockers have favourable effects on the pulmonary vasculature in PAH (reviewed in Ameri et al., 2016). Although β-blocker use in PAH was long regarded as non-beneficial, careful administration in stable PAH without decompensated heart failure was recently shown to have acceptable safety. Several small studies tested the effect of β-blockers on RV function and functional capacity with different outcomes. Thus, larger and longer studies are required to establish which patients (if any) might benefit from specific β-blockers in PAH. Currently, the use of β-blockers is not recommended in patients with PAH unless required for co-morbidities (Perros et al., 2017). More recently, the impact of sympathetic denervation of the pulmonary arteries has been examined; Chinese investigators demonstrated a drop in pulmonary artery pressure using catheter radiofrequency ablation (Chen et al., 2013). Although serious concerns were raised regarding the study design and patient characteristics (Galie & Manes, 2013), a clinical trial is ongoing to examine intravascular ultrasound-directed pulmonary artery denervation in patients on dual oral combination therapy (Treatment of PH 1 Study [TROPHY1]; ClinicalTrials.gov identifier: NCT02835950/NCT02516722).

### 4.1.6 | 5-HT

5-HT (serotonin) has been implicated in the development of PAH since anorexigens, which increase the availability of 5-HT by inducing its release from platelets and inhibiting its reuptake and degradation by MAO, were noted to increase the risk of PAH (Abenhaim et al., 1996). Inhibition of the $5\text{-HT}_{2A}$ and $5\text{-HT}_{2B}$ receptors can inhibit development of PH in mouse models (Delaney et al., 2018; West et al., 2016). Nevertheless, the $5\text{-HT}_{2A}$ and $5\text{-HT}_{2B}$ receptor inhibitor terguride showed no clinical benefit in a Phase 2 study in PAH (Ghofrani et al., 2012), possibly because the $5\text{-HT}_{1B}$ receptor is the most highly expressed 5-HT receptor in the pulmonary arteries in PAH and mediates PASMC proliferation in humans (Lythgoe et al., 2016). However, pre-specified subgroup analysis indicated an improvement of PVR in patients on PAH background therapy with ERAs (Ghofrani et al., 2012). Thus, further studies are planned with a selective inhibitor of tryptophan hydroxylase 1 (TPH1) which is the rate-limiting enzyme in 5-HT biosynthesis. In preclinical PH models, the TPH1 inhibitor significantly reduced PH and showed an additive effect when applied together with ambrisentan, but not tadalafil (Aiello et al., 2017). A clinical Phase 1 study with the TPH1 inhibitor KAR5585 (ClinicalTrials.gov identifier: NCT02746237) showed a good safety profile and a decrease in circulating 5-HT (Paralkar et al., 2017). Furthermore, a trial with the 5-HT uptake inhibitor escitalopram was completed in 2008, but no results were published (ClinicalTrials.gov identifier: NCT00190333). Another trial with the selective 5-HT uptake inhibitor fluoxetine is planned (ClinicalTrials.gov identifier: NCT03638908), although a recent analysis using the “Registry to Evaluate Early and Long-term PAH Disease Management” (REVEAL) showed that incident selective 5-HT uptake inhibitor use was associated with increased mortality and a greater risk of clinical worsening, albeit without adjustment for all confounders (Sadoughi et al., 2013).

### 4.1.7 | Other hormones

Several other hormones have effects on the cardiopulmonary system. One of the long-standing hormones investigated in the field of PAH is vasoactive intestinal peptide (VIP), a peptide hormone that stimulates contractility in the heart and causes dilation of smooth muscles of different organs, including blood vessels. Despite failure of a previous clinical trial with inhaled administration of VIP (Said, 2012), a future study with a s.c. injected, sustained-release analogue of VIP (PB1046) is planned (ClinicalTrials.gov identifier: NCT03315507), because there were serious concerns that the inhaled route of administration may have affected the outcome negatively (Lythgoe et al., 2016). The best examples of endocrine dysfunction in PAH are thyroid disorders, which are common in PAH and associated with poor prognosis (Simonneau et al., 2019).

In summary, there are multiple approaches to balance the hormonal state in PAH. However, despite promising preliminary results, potential beneficial (and non-beneficial) effects of hormones independent of their action on the pulmonary vasculature/RV need to be carefully investigated.

## 4.2 | GPCR pathways

### 4.2.1 | Rho-associated protein kinase

Rho-associated protein kinase (ROCK) belongs to the family of serine–threonine kinases and can be activated via the GTPase RhoA by several cellular receptors, including GPCRs which are stimulated by various vasoactive substances such as angiotensin II or 5-HT. ROCK is involved in many cellular functions, for example, smooth muscle cell contraction, cell migration, and stress fibre formation, and has been implicated in the pathogenesis of PAH (Antoniu, 2012). ROCK inhibitors have shown promising results in animal studies (Antoniu, 2012). However, in a clinical trial, the 6MWD was not improved by the ROCK inhibitor fasudil (Fukumoto et al., 2013). Novel ROCK inhibitors are under development (Vaidya et al., 2017).

### 4.2.2 | Apelin

Apelin is an endogenous vasodilatory and inotropic peptide acting via the G protein-coupled apelin receptor. Apelin is down-regulated in human PAH and can inhibit PH in animal models. Use of apelin for PAH might be challenging due to its short $t_{1/2}$ and systemic vasodilatory effect (Kazemi-Bajestani et al., 2012; Kim, 2014). The effect of apelin infusion on cardiopulmonary performance in healthy volunteers and patients with IPAH was recently investigated in a small clinical trial (ClinicalTrials.gov identifier: NCT01590108). Results are still pending.

### 4.2.3 | Novel preclinical targets

Future approaches may include activation of NO release and inhibition of RhoA/ROCK signalling by activation of the G protein-coupled adenosine $A_{2A}$ receptor (Alencar, Montes, Barreiro, Sudo, & Zapata-Sudo, 2017). Salidroside, an active ingredient isolated from *Rhodiola rosea*, inhibited chronic hypoxia-induced PH and pulmonary arterial remodelling by increasing $A_{2A}$ receptor expression and enhancing $A_{2A}$ receptor-related mitochondria-dependent apoptosis (Huang et al., 2015). Another potential target is the Wnt/planar cell polarity pathway which involves the Frizzled family of GPCRs. Activation of the Wnt/planar cell polarity pathway was recently shown to be required for the establishment of human pulmonary endothelium-pericyte interactions. Loss of Wnt/planar cell polarity signalling could reduce the viability of newly formed vessels in PAH and thus contribute to vascular pruning. Interestingly, mice lacking endothelial expression of Wnt5a showed a similar response to chronic hypoxia as wild-type mice but failed to recover after re-exposure to normoxia (Yuan et al., 2019).

## 4.3 | Ion channels

Ion channels are crucial for cellular calcium homeostasis and thus regulate vasoconstriction and calcium-dependent transcription factors, but they also regulate cytosolic concentrations of other ions (e.g., potassium) which contribute to cellular survival. Dysregulated cell-type-specific ion channels have been extensively described in PAH and include potassium channels, different types of transient receptor potential channels, calcium sensor proteins, and chloride channels (Boucherat et al., 2015; Lambert et al., 2018). Moreover, the identification of novel heterozygous loss-of-function mutations in the KCNK3 gene in PAH has drawn attention to channelopathies as underlying mechanisms (Ma et al., 2013). in vivo pharmacological activation of KCNK3 had beneficial effects in monocrotaline-induced PH, demonstrating therapeutic relevance (Antigny et al., 2016). Very recent studies also suggest a role for glutaminergic NMDA receptors (Dumas et al., 2018). Despite these promising preclinical approaches, clinical trials addressing ion channels are currently lacking in PAH.

## 4.4 | Mitochondrial and metabolic adaptation

### 4.4.1 | Cellular metabolic alterations—The Warburg effect

The Warburg effect has been described in different animal models of PH (Bonnet et al., 2006; McMurtry et al., 2004), and several studies in humans have underlined the relevance of mitochondrial dysfunction and metabolic alterations in PAH (Farha et al., 2016; Rhodes et al., 2017; Xu et al., 2007). As the Warburg effect was originally described in cancer cells, its discovery in PH essentially contributed to the concept of the "cancer-hypothesis" in PAH (Archer et al., 2008). The Warburg effect is characterised by a switch of cellular metabolism from glucose oxidation to glycolysis despite the presence of oxygen and provides metabolic intermediates for macromolecule synthesis. Metabolic intermediates from the tricarboxylic acid cycle (e.g., acetyl-CoA or oxidised nicotinamide adenine dinucleotide [NAD+]) can support epigenetic alterations thereby promoting proliferation (see below).

The picture of mitochondrial alterations in PH is becoming increasingly detailed, including mitochondrial hyperpolarization, dysbalanced mitochondrial fission and fusion, altered mitochondrial calcium handling due to decreased expression of the mitochondrial calcium uniporter and disturbed interaction with the endoplasmic reticulum, increased glutaminolysis and accumulation of mitochondrial heat shock protein 90 (Boucherat et al., 2018; reviewed in detail in Chan & Rubin, 2017, and Culley & Chan, 2018). Most importantly, a causative role for mitochondrial alterations in the development of PH has been supported by the fact that targeting mitochondrial alterations, for example, using **dichloroacetate** (DCA), which promotes glucose oxidation, or the mitochondrial fission inhibitor Mdivi or by inhibiting fatty acid oxidation, inhibited PH in animal models (Chan & Rubin, 2017; Culley & Chan, 2018).

Encouraged by the promising preclinical results, an open-label clinical trial was performed to test the effect of DCA in patients with IPAH (Michelakis et al., 2017). After 4 months of DCA treatment, the average mPAP and PVR decreased and functional capacity increased, but some of the patients did not respond to treatment. Four of the 20 patients withdrew from the study due to neuropathic side effects (all of them from the highest dose group). Detailed analysis showed that lack of treatment response was associated with loss-of-function polymorphisms of specific genes that affect mitochondrial metabolism (**sirtuin 3** [*SIRT3*] and **uncoupling protein 2** [*UCP2*]). Interestingly, mice deficient in UCP2 exhibited PH under baseline conditions (Dromparis et al., 2013; Pak et al., 2013). SIRT3 deficiency has also been connected to spontaneous development of PH (Paulin et al., 2014), but animal studies have not been completely consistent (Waypa et al., 2013). The role of SIRT3 is described in more detail in Section 4.6. Although the DCA trial only showed limited treatment effects, it was an important step to show the feasibility of mitochondrial targeted therapy in PH and to demonstrate the practical use of individualised therapy. Further large-scale studies are needed to investigate the efficacy of DCA in specific patients at different doses. However, DCA may not be optimal for PAH treatment, because of its rapid clearance, highly variable exposure (with individually varying inactivation by GSH transferase zeta 1) and small therapeutic window (Rowlands, 2016), as well as the dose-limiting risk of neuropathy in adults. Further research is necessary to identify novel mitochondrial targets or optimised alternatives to DCA as a promising approach for treatment of PAH.

The concept of mitochondrial dysfunction in PAH has also been applied to the RV, with promising preclinical results (see Chan & Rubin, 2017). However, one must consider that altering mitochondrial function may have opposing effects on the RV and pulmonary vasculature. For example, although UCP2 deficiency is associated with spontaneous PH and RV dysfunction under baseline conditions (Esfandiary et al., 2019; Pak et al., 2013), UCP2-deficient mice show better RV adaptation to increased afterload (induced by pulmonary arterial banding) than wild-type mice (Esfandiary et al., 2019). More details on mitochondrial dysfunction and anti-apoptotic mechanisms were recently summarised by Boucherat, Vitry, et al. (2017).

### 4.4.2 | Systemic metabolic alterations

PAH has been associated with glucose intolerance and insulin resistance. Glucose intolerance was found to be a predictor of mortality in PAH (Belly et al., 2012). A more detailed study recently showed similar glucose intolerance in patients with PAH and controls when matched for the metabolic syndrome, but profound alterations in lipid metabolism and lipid-related insulin resistance (Hemnes et al., 2019). It remains unclear if these alterations contribute to the pathogenesis of PH or are consequences of PH. Nevertheless, optimisation of blood glucose level may have beneficial effects in PAH, although this has not yet entered the guidelines for PAH treatment beyond optimal standard of care for diabetes. Several clinical trials now address this question in PAH and PH due to heart failure with preserved ejection fraction. A Phase 2 clinical trial is investigating the impact of 12 weeks of **metformin** treatment on 6MWD and further functional parameters, RV and left ventricular performance, and several metabolic and hormonal parameters (ClinicalTrials.gov identifier: NCT03617458). Inhibitors of dipeptidyl peptidase 4 (gliptins) which increase **glucagon-like peptide 1** (GLP-1) and insulin release may also be of interest; **sitagliptin** alleviated PH induced by monocrotaline, bleomycin, or hypoxia in rats (Xu et al., 2018). The **GLP-1 receptor** agonist **liraglutide** both prevented and reversed monocrotaline-induced PH, RV hypertrophy, and pulmonary vascular wall remodelling (Lee et al., 2016). The concept of using statins to improve PAH by addressing systemic lipid metabolism has been unsuccessful (Kawut et al., 2011; Zeng et al., 2012).

Another concept identified in the guidelines for PAH treatment is related to iron metabolism. Although the underlying mechanisms are not completely understood, a recent open-label study showed that i.v. iron supplementation in patients with PAH increased exercise endurance capacity, although 6MWD was not changed significantly (Ruiter et al., 2015). This study included only patients without anaemia, and blood Hb content did not change significantly after iron infusion. In this regard, several groups have shown that iron deficiency in the absence of anaemia is common and associated with reduced survival in PAH (Rhodes et al., 2011). Another pilot study demonstrated an increase in 6MWD in patients with PAH and iron deficiency receiving i.v. iron supplementation compared with matched patients without iron deficiency who did not receive iron supplementation. However, Hb levels were also increased in the treated patients (Viethen et al., 2014). A double-blind study of iron infusion in iron-deficient patients with PAH was recently completed, and results are pending (ClinicalTrials.gov identifier: NCT01447628).

## 4.5 | Epigenetic alterations and interaction with metabolic pathways

The role of epigenetics in PAH is a fast-growing field of research. The major epigenetic phenomena include DNA methylation, histone modifications, and modulations of non-coding RNAs (reviewed in Chelladurai et al., 2019; Pullamsetti, Perros, Chelladurai, Yuan, & Stenmark, 2016). Despite encouraging preclinical studies, few of them have reached the clinical stage in PAH yet, mostly because their modulation is often associated with unwanted effects and strategies to therapeutically deliver non-coding RNA mimics or inhibitors to the lungs remain in their infancy (Meloche, Paulin, Provencher, & Bonnet, 2015).

Recently, clinical interest in **histone deacetylase (HDAC)** inhibition in PAH has been rekindled by the discovery that the cytosolic **HDAC6** is involved in both pulmonary arterial remodelling and RV failure (Boucherat, Chabot, et al., 2017). This isoenzyme represents an important pharmacological target for selective inhibition that may reduce the toxicity related to the off-target effects of pan-HDAC inhibitors previously described in PAH (Bogaard et al., 2011). Other HDACs known as Sirtuins are also implicated in PAH. The Sirtuins are NAD+-dependent HDACs regulating important metabolic pathways involved in many biological processes such as cell survival, proliferation, apoptosis, DNA repair, and cell metabolism all of which are critical to PAH development. Consistent with these findings, mice lacking SIRT3, a mitochondrial deacetylase, have increased acetylation and inhibition of many mitochondrial enzymes and complexes, suppressing mitochondrial oxidative metabolism. These mice spontaneously develop PH; a loss-of-function *SIRT3* polymorphism is associated with PAH development in humans (Paulin et al., 2014). The importance of this metabolism–epigenetics axis has been further highlighted by the recent clinical trial results using DCA, a pyruvate dehydrogenase kinase inhibitor known to promote glucose oxidation (see above). The DCA trial showed that functional variants of *SIRT3* and *UCP2* largely influenced the clinical and haemodynamic response to DCA (Michelakis et al., 2017). Although not fully understood, the down-regulation of both **SIRT1** and SIRT3 in PAH might also result from activation of PARP-1 (Meloche et al., 2014), which could cause depletion of NAD+ (SIRT substrate) levels, which inhibits SIRT1 activity (Meloche et al., 2014). Inhibition of **PARP1** in conjunction with standard combination therapy (ERA + PDE5 inhibitor) in an experimental PH model showed greater efficacy than standard combination therapy alone (Meloche et al., 2014), and thus, the U.S. Food and Drug Administration-approved PARP1 inhibitor **olaparib** is under clinical investigation in the Olaparib for PAH study (OPTION; ClinicalTrials.gov identifier: NCT03782818).

The epigenetic/metabolism/DNA damage response axis described above in PAH is very similar to that described in cancer. This cancer theory of PAH (Boucherat, Vitry, et al., 2017) is further reinforced by the implication of the newly described epigenetic reader, **bromodomain-containing protein 4 (BRD4)**, in PAH. Similar to cancer cells in which BRD4 has been shown to promote several oncogenes implicated in PAH pathogenesis, including c-Myc, **B-cell lymphoma 2 (Bcl-2)**, cyclin-dependent kinase inhibitor 1 (p21), cyclin-dependent kinase inhibitor 1B (p27), Runt-related transcription factor 2 (RUNX2), and FOXM1 (Belkina & Denis, 2012), we recently documented that BRD4 is significantly overexpressed in human PAH, accounting for the up-regulation of the oncogenic NFAT, Bcl-2, Survivin, and p21 and triggering the proliferation/apoptosis imbalance in PAH-PASMCs (Meloche et al., 2015; Meloche et al., 2017). BRD4 was similarly up-regulated in PH rat models in which its inhibition improved pulmonary haemodynamics, RV function, and distal pulmonary arterial remodelling. Although these effects were attributed to the modulation of NFAT, Bcl-2, Survivin, and p21, other mechanisms cannot be excluded. The mechanisms accounting for BRD4 inhibitor efficacy in PAH are the subject of numerous published and ongoing preclinical studies. In addition to its effects on the cancer-like phenotype of PASMCs, the inhibition of autoimmune-mediated/inflammatory vascular injuries, RUNX2-mediated pro-calcification processes, and its influence on metabolism and DNA damage are suggested mechanisms of therapeutic intervention by BRD4 inhibitors in PAH. Recently, a clinically available BRD4 inhibitor reversed PH in two independent animal studies potentially through FOXM1 (Van der Feen et al., 2019). Altogether, these findings support a therapeutic role for BRD4 inhibitors in PAH, which is currently being explored in the Apabetalone for PAH Pilot Study (APPRoAcH-p; ClinicalTrials.gov identifier: NCT03655704).

## 4.6 | Oxidative stress-related pathways

Oxidative stress has been implicated in the development of PAH, although animal studies in this regard are inconclusive, depending on the model used (Fulton et al., 2017). Increased mitochondrial ROS release might be more relevant for RV remodelling than PH, at least in the hypoxic mouse model (Pak et al., 2018). Increased levels of markers of oxidatively modified proteins (e.g., nitrotyrosine), fatty acids (e.g., malondialdehyde), and DNA have been detected in lung sections, plasma, or urine of patients with PAH (Bowers et al., 2004; Cracowski et al., 2001; Irodova, Lankin, Konovalova, Kochetov, & Chazova, 2002) but the clinical relevance remains unclear. A small open-label clinical trial with the antioxidant ubiquinol showed a reduction of indirect signs of oxidative stress and right atrial pressure after 3 months of treatment, but no alterations of BNP/6MWD could be detected (Sharp et al., 2014). Non-specific inhibition of ROS may result in disappointing outcomes, because low levels of ROS are necessary to maintain physiological cellular functions.

The pleiotropic effects of ROS on metabolism and inflammation in PH were recently explored. Inhibition of **apoptosis signal-regulating kinase 1 (ASK1**; MAP3K5) a serine/threonine kinase that is activated by oxidative stress, promoting inflammation, ROS production, proliferation, fibrosis, apoptosis, mitochondrial damage, and, under certain contexts, insulin resistance) reduced pathological remodelling and halted progression of PH in rodent models (Budas et al., 2018). Despite these encouraging results, a Phase 2 study (ClinicalTrials.gov identifier: NCT02234141) evaluating the ASK1 inhibitor **selonsertib** did not achieve its primary endpoint in PAH (Boucherat, Provencher, & Bonnet, 2018). Conversely, a Phase 2 clinical trial evaluating **bardoxolone**, an activator of 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-κB, showed significant improvements in exercise capacity in PAH (LARIAT; ClinicalTrials.gov identifier: NCT02036970), albeit mainly in connective tissue disease (CTD)-associated PAH (Oudiz et al., 2017). Thus, a follow-up trial in patients with CTD-PAH was initiated (CATALYST; ClinicalTrials.gov identifier: NCT02657356), and a Phase 3 clinical trial evaluating long-term safety of bardoxolone in patients with PAH is ongoing (RANGER; ClinicalTrials.gov identifier: NCT03068130). Results of a Phase 2 clinical trial evaluating CXA-10, another Nrf2 activator and NF-κB suppressor, are also pending (PRIMEx; ClinicalTrials.gov identifier: NCT03449524).

## 4.7 | Inflammatory mediators

### 4.7.1 | Immune modulators in PAH

Immune modulation is an established concept in PAH, but classic anti-inflammatory drugs such as corticosteroids or **acetylsalicylic acid** have shown beneficial effects only in specific forms of PAH (Sanchez, Sitbon, Jais, Simonneau, & Humbert, 2006) or no beneficial effects (Kawut et al., 2011). However, interest in the field has been revived by an improved understanding of immune regulation in PAH and the notion that perivascular inflammatory infiltrates (macrophages, B-cells, T-cells, and dendritic cells) often precede structural pulmonary vascular remodelling (Tamosiuniene et al., 2011). Histopathological studies have demonstrated the presence of complement system components, autoantibodies, and inflammatory cells (neutrophils) in the vessel lumen, which can bind to the endothelium and may infiltrate the medial muscular layer. The inflammatory infiltrate in the neointimal layer is composed of T- and B-lymphocytes, with macrophages, mast cells, and dendritic cells present in the adventitial layer. Lymphoid follicles, characterised by T-cells, B-cells, and plasmacytoid dendritic cells, are found in the periadventitial space (see Rabinovitch, Guignabert, Humbert, & Nicolls, 2014). Although current knowledge of the immune system is far from complete, increasing evidence suggests that excessive local secretion of inflammatory mediators by pulmonary vascular cells (e.g., IL-1β, IL-6, $LTB_4$, macrophage migration inhibitory factor, leptin, and TNF-α) as well as dysregulated immune responses (innate [through macrophages and monocytes] and adaptive [impaired T-regulatory cell function and T-helper 17 cell immune polarisation]) are major drivers of pulmonary remodelling in PAH with or without autoimmune diseases (see Kuebler, Bonnet, & Tabuchi, 2018; Rabinovitch et al., 2014).

Several immune modulatory approaches have been successfully tested in animal models, including an IL-1 receptor antagonist, IL-6 antibodies, **mycophenolate**, **dexamethasone**, **cyclosporine**, **tacrolimus**, $LTB_4$ pathway inhibitors, and TNF-related apoptosis-inducing ligand (Rabinovitch et al., 2014), but none have yet made the transition to treatment of human PAH beyond CTD-PAH. However, several clinical trials addressing immune modulation are ongoing, including a clinical trial of the anti-CD20 monoclonal antibody **rituximab** (which targets B-lymphocytes) in PAH associated with systemic sclerosis (ClinicalTrials.gov identifier: NCT01086540). Rituximab is often used to treat conditions for which there is a clonal source of autoantibodies. Interestingly, plasmablasts in IPAH displayed clonal expansions similar to those observed in autoimmune disease (Blum et al., 2018). **Anakinra** is a recombinant IL-1 receptor antagonist used to treat rheumatoid arthritis. The safety of anakinra in PAH was recently evaluated in an open-label study. After 14 days of treatment, high-sensitivity C-reactive protein and symptom burden were significantly reduced (Trankle et al., 2019). The recently completed trial of the IL-6 inhibitor **tocilizumab** in PAH (TRANSFORM-UK; ClinicalTrials.gov identifier: NCT02676947) showed no significant change in PVR from baseline at 6 months in the 19 patients who completed the study protocol (published online at https://www.

clinicaltrialsregister.eu/ctr-search/trial/2015-002799-26/results). A Phase 2 open-label extension study with **ubenimex** was recently terminated after the original study failed to demonstrate efficacy (ClinicalTrials.gov identifier: NCT02736149). A different approach uses the endogenous protein elafin which inhibits the neutrophil-derived serine proteases elastase and proteinase-3. Elafin reduced or reversed PH in rats exposed to hypoxia or Sugen/hypoxia respectively (Nickel et al., 2015; Zaidi, You, Ciura, Husain, & Rabinovitch, 2002). Elafin has now progressed to a clinical trial in healthy volunteers (ClinicalTrials.gov identifier: NCT03522935).

#### 4.7.2 | Novel preclinical approaches

Recently, it was reported that fucoidan, a polysaccharidic ligand of the adhesion molecule P-selectin, exhibits anti-proliferative properties and can attenuate hypoxia-induced PH in mice. P-selectin is expressed on endothelial cells and is involved in recruitment of leukocytes and platelets to areas of inflammation and vascular injury respectively (Novoyatleva et al., 2019). Moreover, the damage-associated high mobility group box-1 (HMGB1)—which is secreted from immune cells, activates macrophages as well as lymphocytes, and triggers autoimmunity by binding to the **toll-like receptor 4 (TLR4)**—was shown to be involved in development of PH. Expression of both HMGB1 and TLR4 was elevated in the lungs of patients with PAH. An inhibitor of binding between HMGB1 and TLR4 reduced PH severity and mortality in animal models, even when given to animals with established disease (Goldenberg et al., 2019). Thus, further novel pathways involved in inflammation may be exploited in future for treatment of PH.

### 4.8 | Growth factor receptors

Receptor TKs (RTKs) and the TGFβ superfamily of growth factor receptors are both critically involved in PAH.

#### 4.8.1 | RTKs

RTKs are cell surface receptors for growth factors, cytokines and hormones. RTKs which have been implicated in PAH include PDGF, EGF, FGF, VEGF, and nerve growth factor receptors. Upon activation by growth factors, RTKs dimerise and become autophosphorylated. Binding of factors such as Src and PLCγ leads to activation of several downstream kinases including PI3K/PKB (Akt), MAPKs, PKC, JAK, STAT, and cyclin-dependent kinases (Lemmon & Schlessinger, 2010). Several RTK inhibitors are used to treat different kinds of cancer, including **imatinib**, **dasatinib**, and **nilotinib** (inhibiting the **PDGF receptor**), the multikinase inhibitors **sorafenib** and **sunitinib** (inhibiting the PDGF and **VEGF receptors**), and nilotinib (inhibiting the **BCR-ABL TK**; Lemmon & Schlessinger, 2010). The PDGF receptor inhibitor imatinib was the first RTK used in clinical PAH and the first drug used in PAH to directly target vascular remodelling. However, despite reducing PVR and increasing mean placebo-corrected 6MWD (by 32 m) in a clinical Phase 3 trial (Imatinib in PAH, a Randomised Efficacy Study [IMPRES]), imatinib was not approved for PAH owing to an unfavourable risk–benefit ratio; the imatinib-treated group did not improve time to clinical worsening and showed subdural haematoma in eight patients receiving both imatinib and anticoagulation with vitamin K antagonists (Hoeper et al., 2013). However, treatment with imatinib is currently the only approach beyond the classical, approved and mainly vasodilator therapy that was shown to offer considerable benefit at least in specific patients (Ghofrani et al., 2010; Ghofrani, Seeger, & Grimminger, 2005; Hoeper et al., 2013). Thus, interest in further trials with imatinib and defining groups of patients with an optimal risk–benefit ratio remains high. Moreover, the efficacy of imatinib opened the door to the quest for alternative RTKs with more favourable profiles. Unfortunately, none of the tested RTKs met expectations. A clinical trial with nilotinib was terminated due to severe adverse events (ClinicalTrials.gov identifier: NCT01179737). Exposure to dasatinib was even associated with the development of PAH (Guignabert et al., 2016; Montani et al., 2012). **Nintedanib** was unsuccessful in animal models and when used to treat single patients with PAH on a compassionate basis (Richter et al., 2018). Sorafenib was tested in an open-label study and showed some efficacy (improvement of BNP, uric acid and cardiothoracic ratio of more than 10% from the original value continued for 1 month) in two out of nine patients. However, CO also decreased in most patients treated with sorafenib, raising serious concerns about further trials of sorafenib in PAH (Kimura et al., 2017; Weatherald, Humbert, Guignabert, & Montani, 2017). A similar decrease of CO was observed in a previous open-label 1b trial (Gomberg-Maitland et al., 2010).

#### 4.8.2 | TGFβ superfamily of growth factors

An imbalance in TGFβ/**bone morphogenetic protein (BMP)** signalling has long been known as an important pathogenetic mechanism in PAH, since mutations in *BMPR2*, a TGFβ receptor (TGFβR) subtype, were identified in heritable PAH in 2000 (Lane et al., 2000). Recently, heterozygous mutations of *BMPR2* were identified in 15.3% of patients from a U.K. PAH cohort (76% in familial PAH, 12% in sporadic cases, and 8% in anorexigen-exposed PAH cases; Graf et al., 2018). Decreased **BMPR2** signalling has also been detected in patients with PAH without *BMPR2* mutations. However, attempts to address TGFβ pathways therapeutically have only recently been transferred to the clinic.

TGFβ/BMP signalling is transduced by a combination of different type I (ALK1 to ALK7) and type II (e.g., TGFβR2, BMPR2, and **activin 2A** and **2B receptors**) receptors as well as co-receptors (betaglycan and endoglin). These multicomponent receptors activate canonical downstream signalling via either Smad 2/3 (e.g., through TGFβR2 or activin receptors together with ALK1, ALK2, ALK3, or ALK6) or Smad 1/5/8 (e.g., through BMPR2 or activin receptors together with ALK4, ALK5, or ALK7), or non-canonical signalling via different kinases (e.g., LIM domain kinase 1). Different ligands (e.g., TGFβ, BMPs, and activin) can activate the receptors with different affinity and induce partially opposing effects with regard to development of PAH. While activation of Smad 2/3 signalling (e.g., by TGFβ) results in gene expression promoting PAH, activation of Smad 1/5/8 signalling (e.g., by BMPs) inhibits alterations associated with PAH such as vascular smooth muscle growth (Rol, Kurakula, Happe, Bogaard, & Goumans, 2018). Thus, inhibition or activation of the respective receptor pathway may represent a promising strategy to treat PAH, although balancing the response might be challenging due to overlapping ligand and receptor functions.

The most straightforward therapeutic approach may be activation of BMPR2-Smad 1/5/8 signalling using BMPR2 receptor agonists (e.g., specific ligands) or inhibitors of degradation of BMPR2, such as chloroquine (Thompson & Lawrie, 2017). With regard to BMPR2 activation, tacrolimus (FK506) was recently found to promote BMPR2-Smad1/5/8 signalling by interacting with its pharmacological target, 12-kDa FK506-binding protein (**FKBP12**), thereby removing FKBP12 from all three BMP type 1 receptors (ALK1, ALK2, and ALK3), including those preferred by BMPR2 (ALK1 and ALK3; Sitbon et al., 2019). Tacrolimus prevented the development of PAH in mice with endothelial deletion of BMPR2 and reversed established PAH in two rat models (Spiekerkoetter et al., 2013). Compassionate treatment with tacrolimus showed promising results in single patients with IPAH (Spiekerkoetter et al., 2015) but did not attenuate the progression of PAH in a patient with an ALK1 mutation (Sommer et al., 2019). A Phase 2 clinical trial of tacrolimus in patients with PAH showed favourable safety, but efficacy signals were low (Spiekerkoetter et al., 2017).

Another strategy to address TGFβ signalling involves inhibition of the Smad 2/3 signalling pathway using sotatercept, an activin receptor type 2A fusion protein that acts as a ligand trap. In a preclinical study, sotatercept reversed pulmonary vascular remodelling and restored RV function (Joshi, Liu, Pearsall, Li & Kumar, 2019). A Phase 2 open-label study (SPECTRA; ClinicalTrials.gov identifier: NCT03738150) and a Phase 2 RCT (PULSAR; ClinicalTrials.gov identifier: NCT03496207) are currently recruiting patients with PAH. Primary outcomes will be peak oxygen uptake and PVR respectively.

#### 4.8.3 | Novel preclinical approaches

Smad ubiquitin regulatory factor 1 (SMURF-1) is involved in the degradation of BMPR2 and different Smads and is up-regulated in PAH. Nebulised administration of microRNA-140-5p, shown to target and repress SMURF-1, attenuated PAH in rat models (Rothman et al., 2016). Use of chloroquine to inhibit lysosomal degradation of BMPR2 also restored BMPR2 signalling in vitro and prevented development of monocrotaline-induced PH in rats (Long et al., 2013).

Application of recombinant **BMP9** reversed PH in several animal models, including a mouse model with a human loss-of-function mutation in *Bmpr2* (Long et al., 2015). By contrast, BMP9 deficiency or inhibition prevented development of PH in mice exposed to chronic hypoxia (Tu et al., 2019). It was recently argued that because of the complexity of the BMP system, inhibition of endogenous BMP9 and application of exogenous BMP9 may not be comparable and that inhibition of endogenous BMP9 may inhibit muscularisation but also induce an endothelial pathology similar to that observed in hereditary haemorrhagic telangiectasia (Ormiston, Godoy, Chaudhary, & Stewart, 2019).

Recently, fragile histidine triad (FHIT) was discovered as a novel BMPR2 modifier by a high-throughput screen. FHIT works as a tumour suppressor, and its expression is reduced in patients with PAH. Up-regulation of FHIT by **enzastaurin** reversed PH in the Sugen/hypoxia rat model (Dannewitz Prosseda et al., 2019).

### 4.9 | Transcription factors

#### 4.9.1 | PPAR-γ

**PPAR-γ** belongs to a group of nuclear receptor proteins and functions as a transcription factor. When activated, PPAR-γ improves insulin sensitivity and has anti-inflammatory properties. Both preclinical and human studies have demonstrated the importance of PPAR-γ in PAH. Activation of PPAR-γ signalling by **rosiglitazone** and **pioglitazone** (PPAR-γ agonists used to treat Type 2 diabetes mellitus) inhibited development of PH in animal models (reviewed in Prins et al., 2019). However, despite the promising preclinical results, a clinical trial with pioglitazone (ClinicalTrials.gov identifier: NCT00825266) was terminated early. Furthermore, rosiglitazone has been withdrawn from the market in certain countries due to suspected cardiovascular side effects, and pioglitazone is suspected to cause bladder cancer. Moreover, the doses in preclinical PH studies were significantly higher than the maximal doses used in patients (Prins et al., 2019). Nevertheless, the quest for novel therapeutics exploiting PPAR-γ signalling continues. An inhaled therapy combining sildenafil and rosiglitazone was successfully applied in rats with PH in a recently published study (Rashid, Nozik-Grayck, McMurtry, Stenmark, & Ahsan, 2019).

#### 4.9.2 | HIF and mechanistic target of rapamycin signalling

Hypoxia causes pulmonary vasoconstriction, and hypoxic signalling is also suspected to play a role in PAH. This concept is supported by the finding of decreased expression of prolyl hydroxylase domain-containing protein 2 (PHD2), which inhibits HIF, in PAEC from plexiform lesions, and by the fact that PHD2-deficient mice develop severe, HIF-2α-dependent PH (Dai, Li, Wharton, Zhu, & Zhao, 2016). Accordingly, the HIF-2α translation inhibitor C76 reduced PH in three different rodent models (Dai et al., 2018). At least for hypoxia-induced PH, HIF-1α also seems to be important (Ball et al., 2014).

One strategy to address HIF signalling is via inhibition of **mechanistic target of rapamycin (mTOR)** which increases HIF expression. mTOR can act as a serine/threonine kinase or TK and is activated (for example) by growth factor receptors, but it also integrates signals from high nutrient availability to regulate multiple cellular functions, such as proliferation, motility, survival, and cell cycle progression (e.g., in T-cells). Thus, mTOR inhibitors such as **rapamycin** are used as immunosuppressants, and clinical trials of rapamycin in (for example) pancreatic cancers are currently ongoing. Inhibition of mTOR reduced PH in several animal models (Goncharov et al., 2014; Paddenberg et al., 2007). Moreover, PAH was recently improved in a patient treated with rapamycin for pancreatic cancer (Wessler, Steingart, Schwartz, Harvey, & Schaffer, 2010). Currently, a Phase 1 open-label clinical trial of ABI-009, an albumin-bound version of rapamycin, is recruiting patients with severe PAH (ClinicalTrials.gov identifier: NCT02587325).

### 4.9.3 | Novel preclinical targets

Another transcription factor with high potential as a therapeutic target is **FOXO1**, which integrates several anti-proliferative and pro-apoptotic signalling pathways in PASMC. Total FOXO1 levels were decreased in animal models of PH and in human PAH, and activation of FOXO1 reversed PH in animal models (Savai et al., 2014). Targeting the FOXO1 pathway with **paclitaxel**—which is currently used as a chemotherapeutic agent for different types of cancer—could be a viable strategy for the treatment of PAH. Many other transcription factors have been implicated in the development of PH, including Notch receptor 3, STAT3, large tumour suppressor 1 (central component of the Hippo pathway), myocyte enhancer factor 2, tumour protein p53, kruppel-like factor 4, CCAAT-enhancer binding proteins, RUNX2, activator protein 1, NF-κB, β-catenin, the Twist family basic helix–loop–helix transcription factor 1, and SLUG. Moreover, transcription factor coactivators have also been implicated in the development of PH (see Humbert et al., 2019; Pullamsetti et al., 2016).

## 5 | WHERE TO GO IN FUTURE?

There is consensus that PAH therapy must go beyond targeting primarily vasodilation. Although a wide range of novel targets addressing vascular and RV remodelling have been identified by preclinical studies, the challenge will be prioritising targets and designing clinical trials for successful target validation. The success rate of clinical trial programmes (measured as the proportion of drugs entering clinical development that reach the marketplace) has decreased from 21% in the early 1990s to 16%; about 50% of drugs entering clinical development fail because of lack of efficacy (25% fail because of safety concerns; Lythgoe et al., 2016). In order to prioritise targets, one has to consider the set-up of the preclinical study with regard to limitations of different animal models, species-specific pathways, and drug dose requirements.

How can we overcome the gap between animal studies and clinical trials? Considering the high success rate in animal models, one should ask if the animal models oversimplify the patho-mechanisms. The predominant stimulus in the chronic hypoxic animal model is HIF-1 stabilisation, while the monocrotaline model is largely driven by inflammation. By contrast, human PAH is triggered by more than one stimulus acting over a long time period. Multifactorial pathogenesis and individually varying pathways may hamper disease targeting in individual patients (Lythgoe et al., 2016; Provencher et al., 2018). Thus, the use of several animal models (possibly in a multicenter approach) and animal models induced by more than one hit (e.g., Sugen/hypoxia or BMPR2 knockout plus hypoxia) might be a good step forward for preclinical studies of potential PAH therapies, although animal models reflecting end-stage disease are probably still lacking. The use of mostly very young and often male mice for preclinical studies may also be a limiting factor. Moreover, potential new therapies should be tested on a background of existing PAH therapy, as most patients in clinical trials will be pretreated with approved therapy. Finally, one should consider that many pathways (particularly immunological pathways, Mestas & Hughes, 2004, but also metabolic pathways and distribution of specific vascular receptors and enzymes) differ between mice and humans. Thus, transfer to the human system at any step of research is mandatory and should include more sophisticated models, such as organ culture, cultured lung slices, or induced pluripotent stem cells to derive endothelial cells. Nevertheless, animal models may offer optimal conditions to identify specific pathways that trigger the disease in a particular patient population.

Identifying the patient population which may profit most from a specific treatment ("enrichment") and applying individualised medicine will allow successful targeting of very specific pathways (Sitbon et al., 2019). Great efforts have been made in the past years using genomics, epigenomics, and metabolomics to identify sub-clusters within patient groups (Pulmonary Vascular Disease Phenomics [PVDOMICS]; ClinicalTrials.gov identifier: NCT02980887). Thus, it will be more important for future clinical trials to utilise biomarkers to focus on specific patient subgroups (although this will further limit the number of patients eligible for enrolment). Moreover, detailed patient phenotyping can help to find pathways in common with animal studies and thus prioritise targets. Finally, specific effects of treatment on the pulmonary vasculature and RV have to be taken into account in future.

### 5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan et al., 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Cidlowski et al., 2019; Alexander, Fabbro et al., 2019a, 2019b; Alexander, Kelly et al., 2019a, 2019b).

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## CONFLICT OF INTEREST

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