

Novel and Relevant Mechanistic Pathways

Martin R. Wilkins, MD, FRCP
*Centre for Pharmacology & Therapeutics
 Department of Medicine
 Hammersmith Campus, Imperial College
 London
 London, UK*

Marlene Rabinovitch, MD
*Stanford Children's Health Betty Irene
 Moore Children's Heart Center
 Stanford University School of Medicine
 Stanford, CA*

The current treatments for pulmonary arterial hypertension (PAH) relieve symptoms and may slow the course of the condition but are challenged by the underlying vascular pathology. New treatments are required to arrest and reverse PAH. Here we review a number of exciting candidates based on our understanding of the mechanisms driving the condition.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is characterized by progressive vascular remodeling that increases resistance to blood flow through the lung. The structural remodeling affects primarily precapillary vessels and involves all cellular elements of the vascular wall. The development of new treatments over the last decade has been disappointing, with a high attrition in Phase 2 development.

Increasingly, PAH is recognized as a convergent phenotype, the result of the perturbation of a number of molecular pathways. There is an expectation that focusing on a mechanism-based approach to drug development, where the drug target is "hard-wired" into the biology of the condition, will improve success. This review discusses some key potential drug interventions in context and their stage of development (Figure 1).

TARGETS SUPPORTED BY GENETICS

Bone Morphogenetic Protein Signaling
 Mutations in *BMPR2*, which encodes bone morphogenetic protein receptor type 2 (BMPR-2) in the transforming growth factor- β (TGF- β) signaling pathway, segregate with PAH in families with a history of the condition.^{1,2} This highlights the importance of the BMP-TGF- β signaling pathway in pulmonary vascular biology. *BMPR2* is the most

Anti-inflammatory

IL6 antibody (Phase 2)
 Rituximab (Phase 2)
 Rapamycin (Phase 1)
 Anakinra (Phase 1)

BMP/TGF- β axis

Sotatercept (Phase 2)
 FK506 (Phase 2)

Metabolic targets

DCA (Phase 1)
 Metformin (Phase 2)

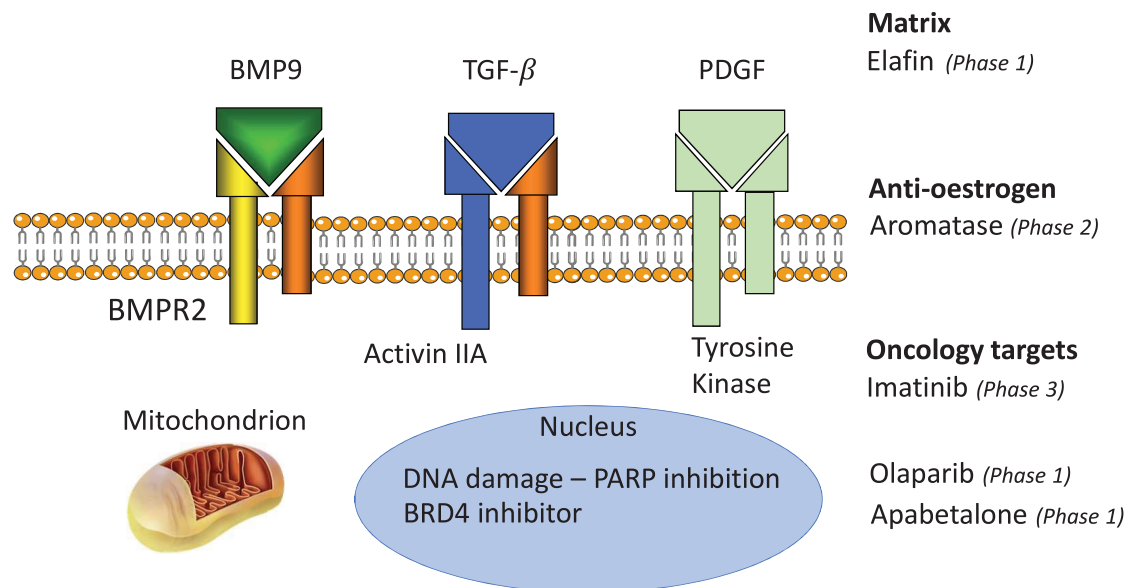


Figure 1: Selected mechanism-based novel therapeutics that have undergone or are planned for human studies. BMPR indicates bone morphogenetic protein receptor; BRD, bromodomain; DCA, dichloroacetate; PDGF, platelet-derived growth factor; PARP, polyADP-ribose polymerase; TGF, transforming growth factor.

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 Correspondence: m.wilkins@imperial.ac.uk
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commonly affected gene associated with PAH, with a frequency of around 15% in idiopathic PAH.³ The variants predict loss of function. Reduced expression of BMPR-2 has been reported in PAH patients without evidence of genetic variant in the encoding gene. The working hypothesis is that BMPR-2 dysfunction creates an imbalance in BMP-TGF- β , prompting interest in either restoring BMPR-2 function or reducing TGF- β activity.

FK506, identified in a screen of 4500 compounds, activates BMPR-2 signaling by removing the inhibitor FKBP12 from the BMPR-2 coreceptor and by inhibiting the phosphatase, calcineurin.⁴ Following encouraging signals in 3 patients with end-stage PAH, a Phase 2 trial identified a dose that increased BMPR-2 expression and was well tolerated.⁵ A more extensive Phase 2 trial with efficacy endpoints is being planned.

A small interfering RNA screen to find gene products that perturb BMPR-2 signaling identified fragile histidine triad,⁶ leading to interest in Enzastaurin. This drug, developed for cancer therapy, increases fragile histidine triad, increases BMPR-2 expression and signaling, and reverses pulmonary hypertension in the Sugen-hypoxia model.⁶ A concern with chronic use has been cognitive impairment, but the efficacious dose may be much lower for PAH than for cancer therapy.

Other strategies for increasing BMPR-2 signaling are directed at suppressing nonsense mutations, using chemical chaperones (eg, 4-phenyl hydroxybutyrate), or inhibiting lysosomal degradation (eg, using hydroxychloroquine) to increase cell surface expression of BMPR-2.⁷ Loss-of-function variants in *GDF2*, which encodes BMP9, a ligand for BMPR-2, underscore interest in the development of BMP9 as a therapy for PAH.⁸ Preclinical studies with BMP9, structurally altered to reduce ectopic bone formation, are well advanced.

An alternative approach, namely, dampening exaggerated activity of TGF- β receptor signaling, is to inhibit the activin IIA receptor using a ligand trap. Sotatercept, developed to treat myelodysplastic syndromes and anemia, has

shown efficacy in abrogating pulmonary hypertension in the Sugen 5416-hypoxia rat and mouse models and in the monocrotaline rat model.⁹ A Phase 2 trial for PAH has shown significant reduction in the primary endpoint of pulmonary vascular resistance and also in secondary endpoints such as 6-minute-walk distance and N-terminal pro-B-type natriuretic peptide as reported in early press releases (ClinicalTrials.gov Identifier: NCT03496207).

Hypoxia-Inducible Signaling Pathway

The Tibetan genome contains variants in *EPAS1*, encoding hypoxia-inducible (HIF)2 α , and *EGLN1*, encoding prolyl hydroxylase 2, that may contribute to physiological adaptation to a hypoxic environment, for example, their relative resistance to hypoxia-induced pulmonary hypertension. The assumption is that the variants in *EPAS1* lead to loss of function in HIF2 α while those in *EGLN1* are associated with gain of function in prolyl hydroxylase 2.¹⁰ Subjects harboring a genetic mutation leading to HIF2 α overexpression show evidence of pulmonary hypertension.^{11,12} Dissociating the effect of these genotypes on hematocrit from a direct effect on pulmonary vascular homeostasis is difficult; a rise in hematocrit increases blood viscosity, which adversely affects pulmonary artery pressure. But genetic manipulation of HIF signaling in rodents suggests that focusing on HIF2 α , which is expressed predominantly in vascular endothelial cells, has promise.

As a transcription factor, HIF2 α is a challenge for small-molecule inhibition, but targeting the hydrophobic cavity in the inner core of the per-ARNT-Sim-B domain offers the opportunity to allosterically disrupt its dimerization to aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF β).¹³ A series of highly selective, orally bioavailable HIF2 α inhibitors have now been developed for exploration in oncology and PT2567 has shown efficacy in rodent models of pulmonary hypertension.¹⁴

Rare Variants in the Human Genome

Whole-genome sequencing has provided a valuable insight into the genetic ar-

chitecture of PAH. Studies to date have revealed or confirmed rare variants in 16 genes.¹⁵ A number, such as *KCNK3*, *TBX4*, *SOX17*, *ATP13A3*, *AQP1*, and *ABCC8*, lie outside the TGF- β signaling pathway, highlighting molecular heterogeneity in PAH. The extent to which these genetically defined targets are druggable remains to be determined.

TARGETS “BORROWED” FROM ONCOLOGY

Pyruvate Dehydrogenase Kinase

Proliferating cells switch their metabolism from oxidative phosphorylation to glycolysis (the Warburg effect), a metabolic switch that appears to confer survival advantage. A key enzyme in this process is pyruvate dehydrogenase, which catalyzes the conversion of pyruvate to acetyl-CoA in mitochondria. Pyruvate dehydrogenase activity is reduced in PAH, in part by activation of pyruvate dehydrogenase kinase, an inhibitor of pyruvate dehydrogenase. Studies in rodent models have shown that a number of strategies designed to restore oxidative phosphorylation also prevent and reverse pulmonary hypertension.¹⁶

Dichloroacetate is a small-molecule inhibitor of pyruvate dehydrogenase kinase, and studies in PAH patients have identified a therapeutic window where there is a reduction in pulmonary artery pressure, coupled with a reduction in lung glucose uptake compatible with improved oxidative phosphorylation, in genetically susceptible patients; specifically, patients without loss-of-function variants in sirtuin 3 or uncoupling protein 2, two proteins that regulate mitochondrial function in a pyruvate dehydrogenase kinase-independent manner.¹⁷

Tyrosine Kinases

The tyrosine kinases are a large family of enzymes that regulate cell growth. Interest with respect to PAH is based on the mitogenic effects of platelet-derived growth factor (PDGF) in pulmonary artery smooth muscle cell culture, the increased expression of PDGF in PAH lungs, and, more compelling, studies of imatinib, a PDGF receptor antagonist, in rodent models and humans.^{18,19} A Phase 3 study (IMPRES) report-

ed an increase in functional capacity (6-minute-walk distance) and improved hemodynamics in PAH patients able to tolerate the drug, but further development was halted because of safety concerns.¹⁹ The demonstration that PAH was associated with the use of dasatinib, a different tyrosine kinase inhibitor, has urged further caution with the use of tyrosine kinase inhibitors as a treatment for PAH.²⁰ But interest in imatinib and PDGF receptor antagonists persists. Clinical studies are underway evaluating low-dose oral imatinib and inhaled-delivery PDGF antagonists in PAH.

Poly-ADP-Ribose Polymerase 1

PAH is associated with DNA damage, evident in pulmonary artery smooth muscle cells isolated from patients.²¹ The expression of polyADP-ribose polymerase 1, a DNA repair enzyme, is increased. Inhibition of polyADP-ribose polymerase 1 with olaparib is approved for the treatment of breast cancer (BRCA)-associated ovarian cancer and BRCA- human epidermal growth factor receptor 2-negative metastatic breast cancer. The premise is that preventing DNA repair promotes cell apoptosis. Following encouraging studies in rodent models, olaparib is under evaluation in an open-label clinical trial in PAH (ClinicalTrials.gov identifier NCT03782818).

Forkhead Box O1

Forkhead box (FOXO) transcription factors are key regulators of cell proliferation. FOXO1 expression is reduced and FOXO1 inactivated by phosphorylation and nuclear exclusion in PAH.²² Paclitaxel increases FOXO1 activity and reduces FOXO1 phosphorylation in pulmonary vascular smooth muscle cells, an effect not replicated by other microtubule stabilizers, and reverses pulmonary vascular remodeling and pulmonary hypertension in rodent models. Clinical studies with an inhaled form of paclitaxel are planned.

Histone Deacetylase and Bromodomain-4

There are 18 histone deacetylases (HDACs) grouped into 4 classes. Inhibition of class I and class II have been explored in cell and animal models,

based in part on the increased expression of HDAC1 and HDAC5 in the lungs of patients with PAH.²³ Concerns about off-target effects, particularly adverse effects on the myocardium, have delayed studies in humans and prompted the search for isoform-selective HDAC inhibitors, in the expectation of a better safety profile.

Increased expression of bromodomain-4, an epigenetic regulator, has also been reported in cells and tissue from PAH patients.²⁴ Increased expression of bromodomain-4 would be expected to promote cell survival and inhibit apoptosis. Apabetalone, an inhibitor of bromodomain-4, is in clinical trials for coronary artery disease and an open label study has been initiated in PAH (ClinicalTrials.gov identifier NCT03655704).

TARGETS SUGGESTED BY EPIDEMIOLOGY

Aromatase

The increased prevalence of PAH in females has naturally raised interest in the role of estrogens. 17 β -estradiol (E2) and/or its metabolite 16 α -hydroxy-estrone have been identified as mediators of PAH.²⁵ Higher circulating E2 levels in men are associated with an increase in the risk of PAH and a shorter 6-minute-walk distance. In postmenopausal women and men, E2 is produced largely from dehydroepiandrosterone-sulfate (DHEA) by the action of aromatase. Interestingly, lower DHEA levels in men are also associated with an increased risk of PAH and a worse prognosis.²⁶

Aromatase is produced in pulmonary arteries in both female animal models of pulmonary hypertension and in women with PAH.²⁷ Support for targeting aromatase comes from studies showing that a single-nucleotide polymorphism in the promoter region of aromatase is associated with a higher level of E2 and increases the risk of PAH in patients with cirrhosis.²⁸ Administration of the aromatase inhibitor anastrozole reduced pulmonary arterial pressures, pulmonary vascular changes, and indexes of right ventricle hypertrophy in experimental pulmonary hypertension.²⁷ Of note, metformin has similar effects via aromatase

inhibition.²⁹ In a proof-of-concept clinical trial, anastrozole significantly reduced E2 levels in patients with PAH.³⁰ It was safe, well tolerated, and improved 6-minute-walk distance but there was interindividual patient heterogeneity in response. It remains to be established whether aromatase inhibition is the optimal approach to inhibiting estrogen. Dual inhibition with fluevestrant has been reported to be more effective in animal models and tamoxifen has been suggested for premenopausal women.³¹

Insulin Resistance

Insulin resistance is common in patients with PAH and associated with a worse prognosis. This has prompted studies of therapeutic agents directed at insulin resistance in PAH. Metformin has been shown to protect and reverse pulmonary hypertension in some but not all experimental rodent models,^{32,33} in part through inhibition of aromatase and estrogen synthesis, as noted above. An experimental medicine study of metformin in PAH (ClinicalTrials.gov Identifier: NCT01884051) and a Phase 2 study to examine its effect on functional capacity are underway (ClinicalTrials.gov Identifier: NCT03617458).

Reduced expression and circulating levels of apolipoprotein E and peroxisome proliferator-activated receptor gamma (PPAR- γ) are components of insulin resistance. Apolipoprotein E internalizes platelet-derived growth factor receptor beta (PDGFR β) and so reduced apolipoprotein E would be expected to enhance PDGFR β signaling.

PPAR- γ has a key role in regulating BMPR-2 and TGF- β signaling pathways in vascular smooth muscle cells. These observations have led to studies demonstrating the reversal of pulmonary hypertension by PPAR- γ agonists rosiglitazone and pioglitazone in rodent models.³⁴

TARGETS IDENTIFIED FROM THE VASCULAR PATHOLOGY

Given the extensive nature of the inflammatory response in PAH, a variety of anti-inflammatory therapies have been proposed. Despite preclinical data,³⁵⁻³⁷ clinical trials with rituximab, which inhibits B cells, and tocilizumab,

which inhibits interleukin 6, have been disappointing. Interest in the mammalian target of rapamycin has led to a safety study with inhaled rapamycin in PAH (ClinicalTrials.gov Identifier: NCT02587325) and a pilot study of Anakinra (IL-1 receptor antagonist) in PAH.³⁸

A novel approach is to inhibit neutrophil elastase. Elevated levels of circulating elastase are attributable to activation of neutrophils and the formation of damaging neutrophil extracellular traps.³⁹ Heightened activity of this enzyme leads to breakdown and loss of elastin and the chemoattractant properties of elastin degradation products or peptides perpetuates inflammation. Inhibition of neutrophil elastase reverses experimental pulmonary hypertension in a rat model induced by monocrotaline.⁴⁰ Further studies in mice and rats indicated that increasing the activity of a naturally occurring elastase inhibitor, elafin, would have the same beneficial properties⁴¹ without the hepatotoxicity of synthetic inhibitors. In addition to inhibition of elastase, elafin has additional favorable properties: it inhibits the proinflammatory transcription factor NFκB and it is an antimicrobial agent. Elafin also promotes signaling through BMPR-2 by increasing the interaction of BMPR-2 with caveolin.⁴² A Phase 1 clinical trial in healthy volunteers has been completed (ClinicalTrials.gov Identifier: NCT03522935) and further preclinical testing will be completed in advance of a Phase 2 clinical trial.

CONCLUSIONS

The exponential increase in PAH research has led to an array of potential new therapies that hold promise as they target biologic mechanisms causing structural changes in the lung circulation. It is anticipated that further advances in genetics will accelerate progress. The discovery of more disease-causing mutations and modifiers as well as treatment-related and PAH-related polymorphisms will help in stratifying patients likely to benefit more from one therapy versus another. Advanced bioinformatic tools are becoming available to better integrate and distil complex epigenetic, meta-

bolic, and proteomic networks, thereby directing us to new therapeutic targets. Advances in cell and structural biology should produce compounds in which the beneficial effects can be better separated from adverse consequences, and in which mitigating a process and restoring balance would be the objective, rather than completely abrogating a biologic pathway. Most important will be the inclusion of multiple biomarkers in clinical trials so that it can be established early on whether the expected disease target was in fact suppressed by the dose and dosing schedule used and, retrospectively, whether there is an explanation for unanticipated detrimental or beneficial off-target effects.

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