Repurposing Medications for Treatment of Pulmonary Arterial Hypertension: What's Old Is New Again

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Pulmonary arterial hypertension (PAH) is a rare but lethal disorder caused by several pathological changes in the pulmonary vasculature. There is endothelial cell dysfunction characterized by exaggerated secretion of vasoconstrictive, pro-proliferative substances, such as endothelin, and impaired release of vasodilatory, antiproliferative molecules, such as nitric oxide and prostacyclin.[1–3] This imbalance contributes to increases in pulmonary artery (PA) smooth muscle cell (PASMC) tone and proliferation.[1–3] Moreover, endothelial cells exhibit metabolic reprogramming with a switch to anaerobic glycolysis.[4] In PASMCs, there is evidence of hypercontractility,[5] proliferation and apoptosis resistance due to genetically[6,7] and epigenetically[8] controlled mechanisms, calcium mishandling,[9–11] metabolic reprogramming,[5,12] and abnormal mitochondrial dynamics.[13,14] Mitochondrial metabolic reprogramming, creating a Warburg metabolic phenotype that promotes proliferation, is also observed in pulmonary vascular fibroblasts.[15] Extracellular matrix (ECM) remodeling promotes PAH by increasing vessel stiffness and thereby altering signaling pathways and inducing metabolic derangements through mechanotransduction.[3,16–19] Finally, there is evidence of a significant inflammatory response with T cell, B cell, and dendritic cell infiltration into the pulmonary vasculature[20,21] and elevated levels of circulating inflammatory cytokines.[20,22,23] These molecular, cellular, and histological changes manifest as reduced pulmonary arterial compliance[24] and elevated pulmonary vascular resistance (PVR) and pulmonary arterial pressures[25] that, in aggregate, augment the workload of the right ventricle.[26] As PAH progresses, the heightened demands on the right ventricle lead to right ventricular hypertrophy (RVH), fibrosis, and metabolic derangements that often culminate in RV failure.[27] RV dysfunction is the strongest predictor of mortality in PAH[28–32] and is the major reason that the median survival with PAH is only 5 to 7 years, both in registries[30–32] and in population-based studies.[33]

Although there have been significant gains in the understanding of the pathophysiology of PAH in recent years, advances in PAH therapeutics have not kept pace, and many promising basic science discoveries have not been tested in patients. Approved PAH medications are predominately pulmonary vasodilators that modulate the endothelin, nitric oxide, and prostacyclin pathways.[34] These therapies primarily address the vasoconstrictive phenotype of PAH, which is the predominant feature in only 5% to 10% of patients[35] or those patients deemed vasoresponders.[36] Although current medications provide benefits to nearly all PAH patients even if they are not vasoresponders, they were approved because they significantly increase exercise capacity (6-minute walk distance [6MWD]), improve quality of life, and/or reduce morbidity[37–45]; however, only epoprostenol confers a clear survival benefit.[46] Consequently, there is an urgent need to develop novel, effective therapies that target additional molecular pathways that drive the pathogenesis of PAH in order to supplement our current treatment options with the hopes of accelerating progress toward a cure.

The strategy of expanding the PAH pharmacopeia by repurposing medications that are used as therapy for other medical conditions is attractive because it may accelerate the development of new therapies and reduce the costs associated with new drug discovery for this orphan disease. In PAH, this consideration is important because, worldwide, many PAH patients do not even have access to currently approved therapies,[47] so discovery of an inexpensive treatment option would very likely have a significant global impact. Available drugs have established safety profiles, so the considerable time and money required to exclude common toxicities and to demonstrate tolerability and safety can be reduced. Nevertheless, even for available agents, the need to confirm dosing and to establish the disease-specific adverse effect profiles cannot be circumvented. Experience suggests that PAH patients may be either more or less sensitive to many drugs.

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For example, PAH patients require higher doses of calcium channel blockers than do patients with angina pectoris due to coronary artery disease.[48] Conversely, PAH patients cannot tolerate the equivalent dose of the tyrosine kinase inhibitor sorafenib[49] that cancer patients do. Nonetheless, repurposing has been effectively implemented in PAH. Sildenafil, a phophodisesterase type 5 inhibitor used for erectile dysfunction,[50] was first tested in an acute treatment protocol in 13 pulmonary hypertension patients and provided a favorable hemodynamic response. Sildenafil administration led to a decrease in mean pulmonary arterial pressure (mPAP) and PVR and an increase in cardiac index.[51] Then, a small placebo-controlled crossover trial conducted in 22 PAH patients showed that sildenafil (dose: 25–100 mg, 3 times/day) increases exercise capacity and cardiac index, calculated using echocardiography, and improves quality of life.[52] These findings provided biological plausibility for the SUPER (Sildenafil Use in Pulmonary Arterial Hypertension) trial, which documented significant increases in 6MWD and reductions in mPAP and PVR after 12 weeks of sildenafil treatment.[40] These findings ultimately led to US Food and Drug Administration approval of sildenafil for PAH.

Preclinical research has identified many molecular pathways that contribute to pathological pulmonary vascular remodeling and RV dysfunction in PAH that have yet to be exploited therapeutically. mutations in BMPR2 (bone morphogenic protein receptor 2), for example, are associated with hereditable PAH,[6,7] and the BMPR2 pathway is downregulated in diverse rodent models of PAH[53,54]; however, no current therapy targets BMPR2. Multiple mechanisms contribute to impaired BMPR2 signaling including inflammation-mediated dysregulation,[55,56] estrogen-induced suppression,[57] autophagosomal degradation,[58] and impaired membrane trafficking of the protein.[59] When these mechanisms are targeted in preclinical studies, BMPR2 signaling is partially restored and reductions in pulmonary vascular disease severity are observed.[60] Moreover, the cancer-like,[61] autoimmune/inflammatory,[20,62] and Warburg metabolic phenotypes[4,13,15,63] that promote vascular obstruction and fibrosis caused by increased proliferation and impaired apoptosis of PASMCs,[12,64] endothelial cells,[4] and fibroblasts[15] can also be inhibited to halt or even reverse pulmonary hypertension in animal studies, and yet none of these pathways are exploited by approved PAH-targeted therapies. Another untapped target in PAH is the right ventricle, where ischemia and fibrosis, relating to impaired angiogenesis and a Warburg metabolic phenotype, contribute to RV dysfunction.[65] Importantly, the metabolic changes and the related RV dysfunction can be partially reversed via pharmacological intervention.[65,66] Moreover, in the RV cardiomyocyte, microtubule remodeling causes mistrafficking and dysregulation of JPH2 (junctophilin 2) and subsequent pathological t-tubule remodeling. This pathway can be rectified with colchicine, suggesting a novel therapeutic target to improve RV function.[67] These are just a few of the numerous molecular pathways in both the pulmonary vasculature and the right ventricle that could be targeted to expand the PAH pharmacopeia to improve outcomes for PAH patients.

In this review, we discuss and evaluate the rigor of the preclinical data that support the notion that 22 medications could potentially be used to target molecular mechanisms involved in the pathogenesis of pulmonary vascular remodeling and RV dysfunction in PAH. We highlight currently available drugs that have clinical safety profiles with preclinical evidence of physiological changes at the whole-animal level. We also discuss the available data from completed and ongoing exploratory clinical trials that are attempting to translate the information gleaned from animal models into therapy for PAH patients. Hopefully, this strategy will more rapidly fill the pipeline of drugs for PAH by identifying new agents that can potentially ameliorate or even cure this orphan disease. Repurposing medications may realize benefits for patients by accelerating the flow of ideas from the bench to the bedside.

Aldosterone Antagonists

Aldosterone is a steroid hormone that binds mineralocorticoid receptors, which are present in multiple tissues, including the heart and pulmonary vasculature. Aldosterone alters gene regulation and promotes a wide array of physiological effects including sodium and water retention, cardiac fibrosis, and activation of the sympathetic nervous system.[68,69] The broad distribution of mineralocorticoid receptors and diverse physiological effects underlies the use of aldosterone antagonists for several clinical indications, including left-sided systolic heart failure,[70,71] systemic hypertension,[72] and refractory ascites in cirrhotic patients.[73] Aldosterone antagonists are generally well tolerated. The most important adverse effect is hyperkalemia, which is more frequently observed in patients also treated with an angiotensin-converting enzyme inhibitor[74] or in patients with chronic kidney disease.[75] Finally, gynecomastia occurs in 6.9% to 10% of patients and can be painful and esthetically problematic in men.[68] However, eplerenone, a newer aldosterone antagonist, does not cause gynecomastia and is effective in treating left heart failure.[71]

Contributing to the biological plausibility of targeting aldosterone in PAH, serum aldosterone levels are elevated in PAH patients and correlate with hemodynamic measures of pulmonary vascular disease. In a study that compared 5 controls with 20 PAH patients, serum levels of aldosterone were significantly higher in PAH patients (control versus PAH: 1200±424 versus 5959±2818 pg/mL, $P<0.02$).[76] Moreover, serum aldosterone levels were positively correlated

with PVR (r=0.72, P<0.02) and transpulmonary gradient (r=0.69, P<0.02) and inversely correlated with cardiac output (r=−0.79, P<0.005).[76] Likewise, plasma and lung aldosterone levels are elevated in the monocrotaline rat (MCT rat) model of PAH.[77]

In PAH, increased serum aldosterone levels dampen activation of nitric oxide synthase in endothelial cells,[77] promote adverse ECM remodeling in response to hypoxia in endothelial cells,[78] and stimulate PASMC proliferation.[79] Finally, aldosterone increases expression of the transcription factor Nedd9 (neural precursor cell expressed developmentally downregulated 9) via inhibition of proteolytic degradation in endothelial cells. Nedd9 then transcriptionally activates *COL3A1* (collagen type III alpha 1 chain)[80] to further promote ECM remodeling.

The preclinical data supporting the use of aldosterone antagonists to counteract mineralocorticoid pathway activation to combat pulmonary vascular disease are robust. Aldosterone negatively regulates endothelin B receptor–mediated nitric oxide production in pulmonary endothelial cells. Aldosterone increases production of reactive oxygen species, which oxidize endothelin receptor B at cysteine 405, an amino acid that lies in the endothelin nitric oxide synthase activating region of the receptor. The oxidation of endothelin receptor B reduces nitric oxide production[77] (Figure 1). Treatment of rats with monocrotaline-PAH with the aldosterone antagonist spironolactone (25 mg/kg per day) beginning at the time of monocrotaline injection increases nitric oxide levels in lung extracts and blunts development of adverse pulmonary vascular remodeling.[77] In a reversal study, spironolactone (25 mg/kg per day) given 14 days after monocrotaline injection significantly reduced PA systolic pressure and PVR index.[77] Eplerenone also slows the development of pulmonary vascular disease. Eplerenone (0.6 mg/g chow), initiated concurrently with exposure to hypoxia (O_2 tension 76 mm Hg for 21 days) in the Sugen-5416 (SU-5416) hypoxia rat model, reduces PA systolic pressure.[77]

Another pathological mechanism by which excess aldosterone promotes pulmonary vascular disease is ECM remodeling. In human PA endothelial cells, hypoxia enhances c-Fos/c-Jun binding to the proximal AP1 (activator protein 1) site of the promoter region of StAR (steroidogenic acute regulatory protein) and increases StAR expression.[78] StAR promotes aldosterone synthesis, which in turn induces transcription of CTGF (connective tissue growth factor), collagen III, and MMP2 (matrix metalloprotease 2) and MMP9.[78] In a series of in vivo experiments distinct from those described in the previous paragraph, treatment of SU-5416 hypoxia rats with eplerenone (0.6 mg/g chow for 21 days) starting at the time of SU-5416 injection reduces CTGF and collagen III levels in the pulmonary vasculature and lessens the severity of experimental PAH.[78] In a reversal study, spironolactone (25 mg/kg per day) given 14 days after SU-5416 for 7 days at hypoxia and continued for 16 to 17 days in normoxia reduces RVH, mPAP, and right atrial pressure.[78]

Aldosterone also promotes PASMC proliferation. Aldosterone increases the abundance of phosphorylated $p70^{S6K}$ (70-kDa ribosomal S6 kinase), the active form of the major downstream effector kinase of mTORC1 (mammalian target of rapamycin complex 1), through a mechanism dependent on both Akt[79] and the mTORC1 subunit Raptor[79] in cultured PASMCs. The activation of mTORC1 promotes proliferation and apoptosis resistance of cultured PASMCs (Figure 2).[79] When administered in a preventative manner, spironolactone reduces phosphorylated $p70^{S6K}$ expression in the pulmonary vasculature in MCT rats.[79] Furthermore, combining spironolactone and a small interfering RNA targeting Raptor prevents pulmonary vascular remodeling in MCT rats.[79] In a regression protocol, spironolactone plus small interfering RNA to Raptor reverses pulmonary hypertension in SU-5416 hypoxia rats.[79] Using a scoring system modified from Provencher et al,[81] the scientific rigor score is 4 (Table 1) for the preclinical data supporting the use of aldosterone in PAH.

The impact of spironolactone in PAH is currently being investigated in 2 ongoing clinical trials. The CAPS-PAH (Combination Ambrisentan Plus Spironolactone in Pulmonary Arterial Hypertension Study) is a single-center, double-blind, placebo-controlled, crossover study that will investigate whether addition of spironolactone to ambrisentan alters exercise capacity in 30 PAH patients (ClinicalTrials.gov identifier NCT02253394). PAH patients on ambrisentan for >90 days who are New York Heart Association (NYHA) functional class II or III will be randomized to 50 mg of spironolactone daily or placebo for 90 days and then will undergo testing. After a 21-day washout period, patients will cross over to the other arm for another 90 days of treatment, followed by a repeat assessment. The primary end points are change in 6MWD and maximal oxygen consumption. Secondary outcomes will include estimated cardiac output and RV function using echocardiography, biomarkers of RV failure (NT-pro-BNP [N-terminal probrain natriuretic protein], IL6 [interleukin 6], troponin, and collagen III), and quality of life.

Concurrently, a multicenter, double-blind, randomized, placebo-controlled trial will also examine whether treatment with spironolactone alters outcomes in 70 PAH patients (ClinicalTrials.gov identifier NCT01712620). Patients in NYHA functional classes I to III who are either on stable PAH-specific vasodilator therapy for 4 weeks or treatment-naïve before enrollment will be randomized to placebo or spironolactone (25 mg daily for 7 weeks and, if tolerated, increased to 50 mg daily during week 8). The study will last for 24 weeks with the primary end points being change in 6MWD and clinical worsening. Secondary end points will include change

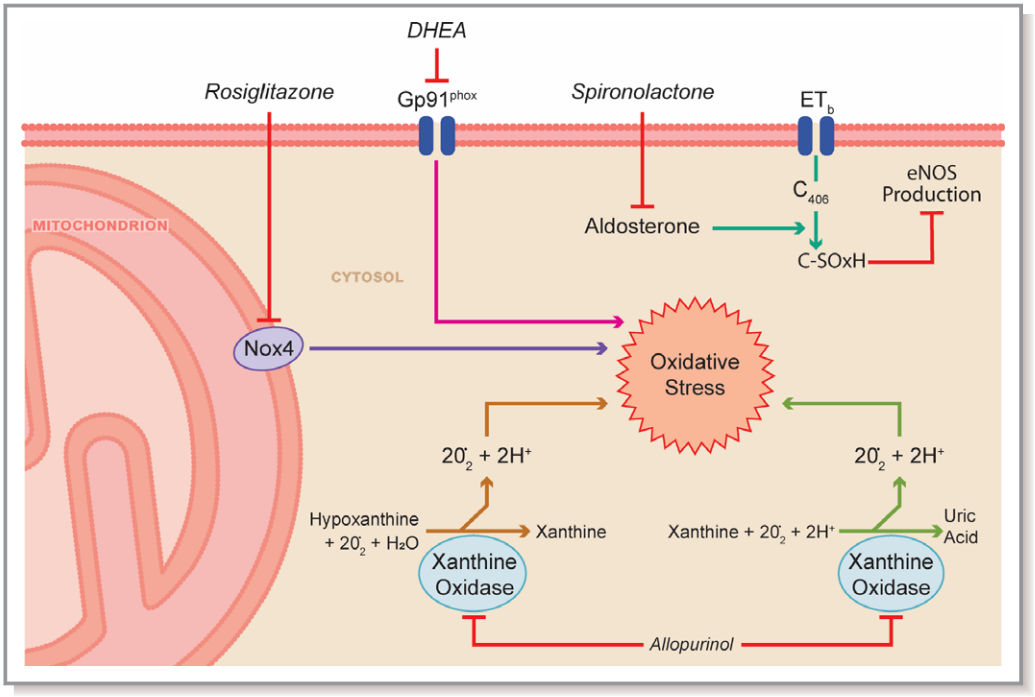

Figure 1. Spironolactone, allopurinol, DHEA, and rosiglitazone combat oxidative stress. DHEA indicates dehydroepiandrosterone; eNOS, endothelial nitric oxide synthase; ET, Endothelin-b receptor; Nox4, NADPH oxidase 4.

in placebo-corrected maximal oxygen consumption, RV function (quantified by cardiac magnetic resonance imaging [MRI]), and markers of inflammation. Finally, discontinuation rates due to adverse effects including hyperkalemia and gynecomastia will be recorded.

In summary aldosterone antagonists could combat endothelial dysfunction, prevent ECM remodeling, and slow PASMC proliferation in PAH. The 2 ongoing clinical trials will determine whether aldosterone antagonists are tolerable and effective in PAH.

Allopurinol

Oxidative stress, including an increase in reactive oxygen species formation, is implicated in the pathogenesis of pulmonary vascular remodeling.[82] Xanthine oxidase catalyzes the transformation of hypoxanthine to xanthine and then to uric acid with the associated production of 4 superoxide anions.[83] Thus, xanthine oxidase is potentially a major regulator of cellular oxidative stress (Figure 1).[84] A pathological role for xanthine oxidase in PAH is suggested in several human studies. In a study of 99 PAH patients, the natural logarithmic transformation of serum uric acid is positively correlated with right atrial pressure (r=0.64, P<0.001).[85] Higher serum levels of uric acid are associated with lower 6MWD and higher mortality in a study of 29 PAH patients.[86] Furthermore, xanthine oxidase activity is elevated in the serum of PAH patients compared with control participants (5201±2836 [n=31] versus 2424±1419 [n=6] arbitrary units, P=0.026).[87] In lung extracts of PAH patients, expression of the oxidative stress markers 8-hydroxyguanosine and nitrotyrosine are increased.[88] Mass spectrometry reveals elevated levels of 5-hydroxyeicosatetraenoic acid, the oxidized product of 5-oxo-eicosatetraenoic acid, in lungs of PAH patients who had not been treated with prostacyclin.[88] Moreover, expression of the antioxidant enzyme SOD_2 (superoxide dismutase), which catalyzes breakdown of superoxide anion to the less toxic H_2O_2,[89] is reduced in PAH lungs.[88] SOD_2 downregulation (in PAH patients and experimental PAH) was independently confirmed and shown to result from an epigenetic mechanism mediated by DNMT1 (DNA methyltransferase 1) and DNMT3b. Methylation of the promoter of the SOD_2 gene reduces SOD_2 protein levels and decreases H_2O_2, which activates HIF-1α (hypoxia-inducible factor 1α), creating a state of pseudohypoxia (normal oxygen tension but activation of hypoxic signaling pathways).[8] Interestingly, this pathological process can be reversed by the DNMT inhibitor decitabine, which is used to treat patients with myelodysplastic disorders.[90]

Allopurinol, a xanthine oxidase inhibitor, is used to prevent gout[91] and nephrolithiasis caused by hyperuricosuria.[92] Allopurinol is well tolerated for extended periods under these conditions. However, renal dysfunction increases the risk of side effects including gastrointestinal discomfort, lung toxicity, epidermolysis syndrome, and hypersensitivity syndrome.[93] Two preclinical studies have examined the utility of allopurinol in pulmonary vascular disease.

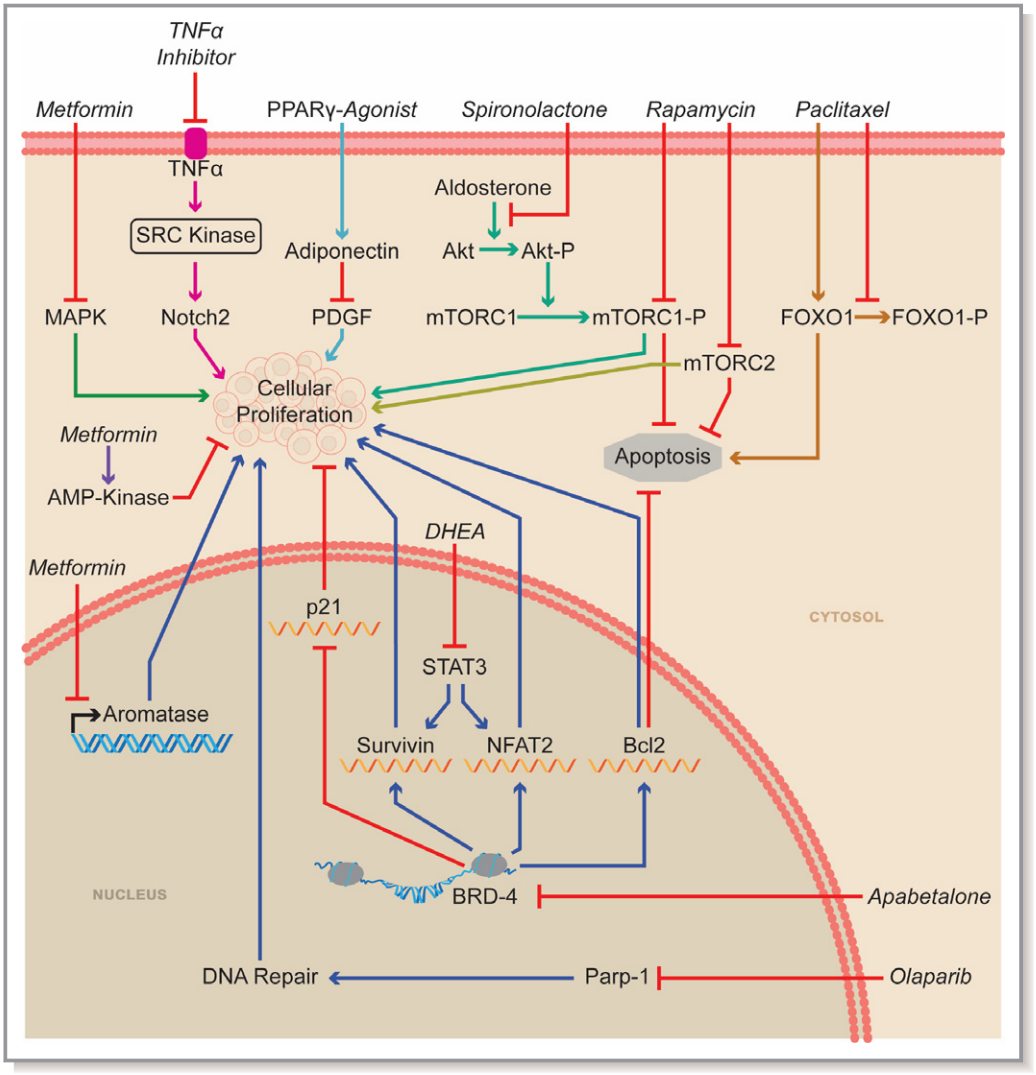

Figure 2. Multiple pathways can be inhibited to alter the proliferation/apoptosis balance of pulmonary artery smooth muscle cells. Bcl-2 indicates B cell lymphoma 2; BRD-4, bromodomain-containing protein 4; FOXO1, forkhead box protein O1; MAPK, mitogen-activated protein kinases; mTORC, mammalian target of rapamycin complex; NFATC2, nuclear factor of activated T cells 2; P, phosphorylation; Parp-1, poly(ADP-ribose) polymerase 1; PDGF, platelet derived growth factor; PPAR-γ, peroxisome proliferator-activator-γ; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α.

Rats exposed to hypoxia (10% oxygen for 7 or 21 days) have elevated levels of PCOOH (phosphatidylcholine hydroperoxide), a marker of oxidative stress that reflects increased xanthine oxidase activity.[94] Treating hypoxic rats with allopurinol (50 mg/kg every 12 hours starting the day before hypoxia exposure) decreases PCOOH levels and blunts adverse pulmonary vascular remodeling and reduces RVH.[94] Likewise, neonatal rats exposed to hypoxia (13% O_2 from birth for 14 days) have increased serum and lung xanthine oxidase activity,[95] and allopurinol (50 mg/kg per day starting the first day of hypoxia) normalizes xanthine oxidase activity and reduces RVH and adverse pulmonary vascular remodeling.[95]

No clinical trials are currently investigating the use of allopurinol in PAH. The ease of administration and favorable side-effect profile suggests that a trial of allopurinol in PAH is feasible. However, human doses would need to be much lower than those used in rodent studies (Table 2),[96] and the rigor of the preclinical studies is low, with a score of 2 (Table 1).

Anakinra

As discussed, substantial evidence shows that inflammation plays a role in PAH pathogenesis. Serum levels of the inflammatory cytokine IL1, which promotes IL6 synthesis (Figure 3), are increased in PAH patients.[22,23] Moreover, IL1 mRNA levels are elevated in the lungs of MCT rats.[97] Furthermore, administration of IL1 to BMPR2 (bone morphogenetic protein receptor type 2) R899X transgenic mice

produces a more severe PAH phenotype.[98] In aggregate, these findings provide evidence of a direct adverse effect of IL1 and inflammation in the pathogenesis of PAH. Anakinra is a recombinant IL1 receptor antagonist that can be used to treat rheumatoid arthritis[99] and recurrent pericarditis.[100] Anakinra is safe, but adverse side effects include headache, vomiting, injection-site irritation, and increased risk of infection due to the immunosuppressive actions.[99]

IL1 antagonism is beneficial in rats with PAH induced by monocrotaline but not in rats with chronic hypoxic pulmonary hypertension induced by hypobaric hypoxia (simulated altitude of 16 000 ft [4877 m]). MCT rats have increased mRNA levels of IL1 and IL1 receptor in lung extracts, whereas hypoxic rats do not.[97] Use of a purified recombinant human IL1 receptor antagonist (2 mg/kg twice a day starting at the time of monocrotaline injection or hypoxia for 2 weeks) reduces mPAP and RVH in MCT rats at the 3-week time point.[97] This approach is associated with a reduction in lung IL1 mRNA levels. In contrast, chronic hypoxic rats experienced no benefit with the IL1 antagonist.[97] The differences in phenotype, with much greater lung inflammation in MCT rats, likely explains the divergent effects. Thus, IL1 antagonism appears to be effective in inflammatory, preclinical PAH models.

The safety of anakinra in PAH was recently reported in a single-arm, open-label study of 6 PAH patients. Patients with connective tissue–associated, HIV, portal hypertension, or schistosomiasis-associated PAH were excluded. In this small study, all patients had evidence of RV dysfunction, as defined by RV diastolic diameter >4.3 cm, fractional area change <35%, and/or tricuspid annular plane systolic excursion ≤ 1.5 cm, and NYHA class II or III symptoms despite optimal therapy. Patients received 100 mg of anakinra daily for 14 days by subcutaneous injection.[101] After 14 days of treatment, high-sensitivity C-reactive protein and symptom burden, as quantified by the Minnesota

Table 1. Numerical Score of Preclinical Rigor of Potentially Repurposed Medications

Drug	Number of PAH Models Used	Regression Evaluated*	Human Tissue/Cells Evaluated*	Randomization Specified*	Power Calculation*	Multiple Publications Demonstrating Efficacy*	Male and Female Sex*	Long-Term Safety Evaluation*	Total Score
Aldosterone antagonist	2	1	1	0	0	1	0	0	5
Allopurinol	1	0	0	0	0	1	0	0	2
Anakinra†	2	0	0	0	0	0	0	0	2
Anastrozole	4	1	1	0	0	0	1	0	7
Apabetalone	1	1	1	1	0	0	0	0	4
β-Adrenergic blockers	2	1	0	0	0	1	0	0	4
Chloroquine	1	1	0	0	0	0	0	0	2
Colchicine	1	1	0	0	0	1	0	0	3
DHEA	3	1	1	0	0	1	0	0	5
Dichloroacetate	5	1	1	1	0	1	1	1	11
Metformin	4	1	1	0	0	1	1	0	8
Nab-rapamycin	2	1	0	1	0	1	1	0	6
Olaparib	2	1	1	1	0	0	0	0	5
Paclitaxel	2	1	1	0	0	0	0	0	4
Ranolazine	2	1	0	0	0	1	0	0	4
Rituximab†	1	1	0	0	0	0	0	0	2
Rosiglitazone/pioglitazone	4	1	1	0	0	1	1	0	8
Tacrolimus	3	1	1	0	0	0	0	0	5
Tocilizumab	2	1	1	0	0	0	0	0	4
Trimetazidine	1	1	0	0	0	0	0	0	2
TNF-α inhibitor	2	1	1	1	1	1	0	0	7
Verteporfin	1	0	1	0	0	0	0	0	2

DHEA indicates dehydroepiandrosterone; PAH, pulmonary arterial hypertension; TNF-α, tumor necrosis factor α.
*1 = yes, 0 = no.
†Indicates a molecule with similar mechanism of action was used in preclinical studies.

Table 2. Summary of Preclinical Results of Potentially Repurposed Drugs for PAH

Drug	Mechanism of Action	Downstream Consequence	In Vivo Effects	Animal Model Used	Animal Model Dose	Equivalent Human Dose*	Maximal Daily Dose in Clinical Practice
Aldosterone antagonist	Inhibition of aldosterone signaling	1. Increased nitric oxide levels in the PV 2. Reduced ECM remodeling in the PV 3. Inhibition of mTORC1 signaling leading to reduced PASMC proliferation	1.Blunted PV remodeling 2. Reduced RVH	MCT SU-5416 hypoxia	Spironolactone (25 mg/kg/d) Eplerenone (0.6 mg/g chow)	Spironolactone: 4.0 mg/kg/d Eplerenone: 0.1 mg/g food	Spironolactone: 200 mg Eplerenone: 100 mg
Allopurinol	Xanthine oxidase inhibitor	1. Reduced PCOOH levels 2. Normalization of xanthine oxidase activity 3. Reduction in overall oxidative stress	1.Blunted PV remodeling 2. Reduced RVH	Hypoxic adult and neonatal rats	50 mg/kg/d 50 mg/kg every 12 h	8.1 mg/kg/d 8.1 mg/kg every 12 h	300 mg
Anakinra	Block inflammatory cytokine IL1	1. Reduced IL1 mRNA in lungs 2. Reduced macrophage infiltration into pulmonary vasculature	1. Blunted PV remodeling in MCT rats 2. Reduced RVH in MCT rats	MCT Hypoxia	Anakinra not used in preclinical study	Anakinra not used in preclinical study	100 mg
Anastrozole	Inhibitor of estrogen signaling	1. Increased BMPR2 signaling 2. Increased expression of PPAR-γ 3. Increased expression of CD36 4. Increased insulin sensitivity 5. Reduction in PASMC proliferation	1. Blunted PV remodeling 2. Reduced RVH	Hypoxic rats Hypoxic mice SU-5416 hypoxia BMPR2 R899X mice	0.03–3 mg/kg/d	0.005–0.5 mg/kg/d	1 mg
Apabetalone†	BRD-4 inhibitor	1. Reduced levels of oncogenic proteins NFATC2, Bcl-2, and survivin 2. Increased expression of p21 3. Reduction in PASMC proliferation	1. Blunted PV remodeling 2. Reduced RVH	SU-5416 hypoxia	Apabetalone not used in preclinical study	Apabetalone not used in preclinical study	300 mg
β-Adrenergic blockers	Counteract excessive sympathetic nervous system activation in right ventricle and pulmonary vasculature	1. Normalization of β-adrenergic signaling in the right ventricle 2. Increased SERCA2a mRNA levels	1. Blunted PV remodeling 2. Decreased RV fibrosis 3. Improved RV function 4. Augmented exercise capacity 5. Improved survival	MCT, SU-5416 hypoxia	Arotinolol (0.25 mg/kg/d) Bisoprolol (10 mg/kg/d) Carvedilol (15 mg/kg/d)	Arotinolol (0.04 mg/kg/d) Bisoprolol (1.6 mg/kg/d) Carvedilol (2.4 mg/kg/d)	Arotinolol: NA, Bisoprolol: 10 mg Carvedilol: 100 mg
Chloroquine	Inhibitor of lysosomal degradation	1. Increased BMPR2 signaling via reduction in lysosomal degradation 2. Reduction in PASMC proliferation	1. Blunted PV remodeling 2. Reduced RVH	MCT	50 mg/kg/d	8.1 mg/kg/d	2.3 mg/kg
Colchicine	Anti-inflammatory and normalization of JPH2 levels via microtubule depolymerization	1. Reduction in PASMC proliferation 2. Restoration of structure and function of T-tubules in RV cardiomyocytes	1. Reduced PV remodeling 2. Reduced RVH 3. Improved RV function 4. Enhanced exercise capacity	MCT	1.0 mg/kg/d for 5 d 0.5 mg/kg 3 times/wk	0.16 mg/kg for 5 d 0.08 mg/kg 3 times/wk	2.4 mg

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Table 2. Continued

Drug	Mechanism of Action	Downstream Consequence	In Vivo Effects	Animal Model Used	Animal Model Dose	Equivalent Human Dose*	Maximal Daily Dose in Clinical Practice
DHEA	Inhibits STAT3 which reduces NFATC2 and survivn and increases BMPR2	1. Reduction in PASMC proliferation 2. Increased PASMC apoptosis 3. Increased BMPR2 signaling	1. Reduced PV remodeling 2. Reduced RVH 3. Improved RV function 4. Enhanced exercise capacity	MCT, SU-5416 hypoxia	10 mg/kg/d 30 mg/kg every other day 1% in food	1.6 mg/kg/d 4.8 mg/kg every other day 0.16% in food	100 mg
Dichloroacetate	Counteract Warburg metabolic effect via PDK inhibition	1. Improved glucose oxidation 2. Reduced PASMC proliferation 3. Increased PASMC apoptosis 4. Increased potassium channel levels 5. Depolarization of mitochondria	1. Reduced PV remodeling 2. Improved RV function 3. Enhanced RV contractility 4. Reduced RVH 5. Increased exercise capacity 6. Improved survival	Hypoxic rats MCT SU-5416 FHR PAB rats	70–80 mg/kg/d 0.75 g/L drinking water	11.3–12.9 mg/kg/d 0.12 g/L of drinking water	25 mg/kg
Metformin	Inhibitor of MAPK activation, inhibitor of aromatase transcription, augments AMP activation	1. Reduced PASMC proliferation 2. Reduced PASMC contractility 3. Reduced RV lipid deposition	1. Reduced PV remodeling 2. Reduced RVH	Hypoxic rats MCT SU-5416 hypoxia BMPR2 R899X	100 mg/kg/d 25 g/kg of high-fat chow	16.1 mg/kg /d 4.0 g/kg chow	2550 mg
Nab-rapamycin	Inhibitor of mTORC1 and mTORC2	1. Reduced PASMC proliferation 2. Increased PASMC apoptosis	1. Reduced PV remodeling (dose dependent) 2. Reduced RVH (dose dependent)	MCT Hypoxic mice	Nab-rapamycin not used in preclinical study	Nab-rapamycin not used in preclinical study	100 mg/m^2
Olaparib	Inhibitor of PARP1	1. Reduced PASMC proliferation 2. Increased PASMC apoptosis	1. Reduced PV remodeling 2. Reduced RVH	MCT SU-5416	6 mg/kg/d	0.97 mg/kg/d	800 mg
Paclitaxel	FOXO1 Activator	1. Reduced PASMC proliferation 2. Increased BMPR2 signaling 3. Increased PASMC apoptosis	1. Reduced PV remodeling 2. Reduced RVH 3. Improved RV function	SU-5416 Hypoxia MCT	5–7 mg/kg/wk 1 mg/kg/wk aerosolized	0.8–1.1 mg/kg/wk 0.16 mg/kg/wk aerosolized	225 mg/m^2 every 3 to 4 wks
Ranolazine	Reduction of FAO and enhancement of glucose oxidation (by activating Randle cycle)	1. Reduced Glut1 and HK1 mRNA levels 2. Increased RV glucose oxidation 3. Increased ATP production 4. Decreased FAO	1. Reduced RVH 2. Improved RV function 3. Decreased RV fibrosis 4. Reduced risk of arrhythmias 5. Increased exercise capacity	PAB rats MCT	20 mg/d 0.25–0.5% in chow	3.2 mg/d 0.04–0.08% in chow	2000 mg
Rituximab†	Anti-inflammatory via blocking of CD20	1. Reduced IL6, HIF-1α, and VEGF 2. Decreased PASMC proliferation	1. Reduced PV remodeling 2. Reduced RVH	Ovalbumin immunization plus SU-5416 rats	Rituximab not used in preclinical study	Rituximab not used in preclinical study	1000 mg every 2 wk

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Table 2. Continued

Drug	Mechanism of Action	Downstream Consequence	In Vivo Effects	Animal Model Used	Animal Model Dose	Equivalent Human Dose*	Maximal Daily Dose in Clinical Practice
Rosiglitazone/ pioglitazone	PPAR-γ activators	1. Increased adiponectin levels 2. Reduced NOX4 levels 3. Reduced PASMC proliferation 4. Improved mitochondrial organization 5. Induced FAO genes 6. Improved FAO efficacy in cardiomyocytes	1. Reduced PV remodeling 2. Reduced RVH 3. Improved RV function	ApoE knockout mice Hypoxic rats Hypoxic mice SU-5416 rats	Rosiglitazone (8–10 mg/kg/d) Pioglitazone (20 mg/kg/d)	Rosiglitazone (1.3–1.6 mg/kg/d) Pioglitazone (3.2 mg/kg/d)	Rosiglitazone: 8 mg Pioglitazone: 45 mg
Tacrolimus	Calcineurin inhibitor	1. Sequestered FK-binding protein 2 from BMPR1 receptors 2. Increased BMPR2 signaling 3. Improved endothelial function 4. Reduced PASMC proliferation	1. Reduced PV remodeling 2. Reduced RVH	BMRP2 endothelial knockout mice MCT SU-5416 hypoxia	0.05 mg/kg/d	0.008 mg/kg/d	0.6 mg/kg
Tocilizumab†	Inhibit inflammatory cytokine IL6	1. Reduced STAT3 activation 2. Induced PASMC apoptosis	1. Reduced PV remodeling 2. Reduced RVH	MCT SU-5416 hypoxia	Tocilizumab not used in preclinical study	Tocilizumab not used in preclinical study	800 mg every 4 wk
Trimetazidine	Reduce FAO and enhance glucose oxidation (by activating Randle cycle)	1. Reduced Glut1 and HK1 mRNA levels 2. Increased RV glucose oxidation 3. Increased ATP production 4. Decreased FAO	1. Reduced RVH 2. Improved RV function 3. Improved exercise capacity	PAB rats	0.7 g/L of drinking water	0.11 g/L of drinking water	70 mg
TNF-α inhibitor	Anti-inflammatory via blocking of TNF-α signaling	1. Increased BMPR2 signaling 2. Decreased NOTCH2 expression 3. Reduced PASMC proliferation	1. Reduced PV remodeling 2. Reduced RVH	MCT SU-5416	Etanercept: 2.5 mg/kg twice weekly	0.4 mg/kg twice weekly	Etanercept: 100 mg twice weekly
Verteporfin	Inhibitor of YAP-induced glutaminolysis	1. Decreased lysyl oxidase activity 2. Reduced glutaminase activity 3. Reduced pulmonary arteriolar stiffness 4. Decreased PASMC proliferation	1. Reduced PV remodeling 2. Reduced RVH	MCT	25 mg/kg/d	4.0 mg/kg/d	6 mg/m^2 every 3 mo

ApoE indicates apolipoprotein E; Bcl-2, B cell lymphoma 2; BMPR, bone morphogenic protein receptor; BRD-4, bromodomain-containing protein 4; ECM, extracellular matrix; FOXO1, forkhead box protein O1; FHR, Fawn hooded rat; Glut1, glucose transporter 1; HIF-1α, hypoxia-inducible factor 1α; HK1, hexokinase 1; JPH2, junctophilin 2; IL, interleukin; MAPK, mitogen-activated protein kinase; MCT, monocrotaline; mTORC, mammalian target of rapamycin complex; NA, not available; NFATC2, nuclear factor of activated T cells 2; NOTCH2, notch 2; PAB, Pulmonary artery banded; PAH, pulmonary arterial hypertension; Parp-1, poly(ADP-ribose) polymerase 1; PASMC, pulmonary artery smooth muscle cell; PCOOH, phosphatidylcholine hydroperoxide; PPAR-γ, peroxisome proliferator-activator γ; PV, pulmonary vasculature; RV, right ventricular; RVH, right ventricular hypertrophy; SERCA2a, sarco/endoplasmic reticulum Ca^{2+}-ATPase; STAT3, signal transducer and activator of transcription 3; SU-5416, Sugen-5416; VEGF, vascular endothelial growth factor; YAP, Yes-associated protein.

*Indicates human dose was calculated via differences in body surface area.[96]

†Indicates a molecule with similar mechanism of action was used in preclinical studies.

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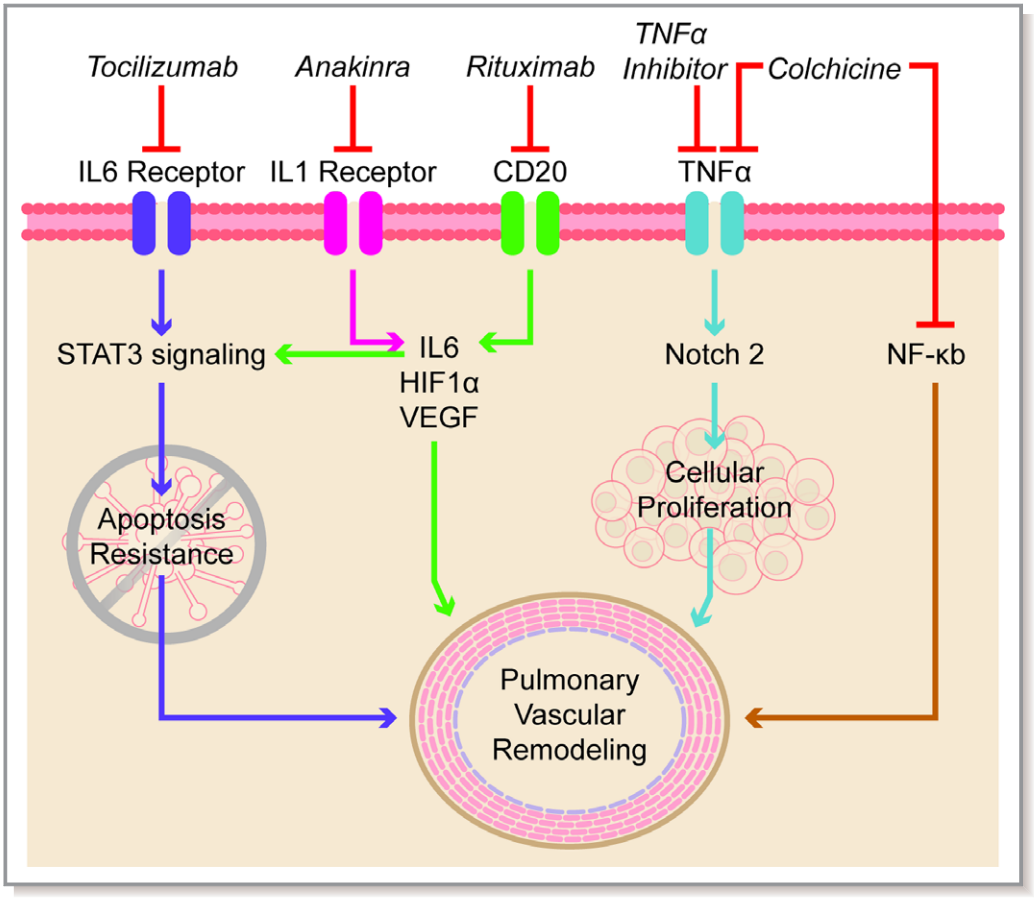

Figure 3. Proinflammatory pathways that can be targeted for treatment of pulmonary arterial hypertension. HIF-1α indicates hypoxia-inducible factor-1α; IL, interleukin; NF-κB, nuclear factor κB; NOTCH2, notch 2; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

Living with Heart Failure Questionnaire, were significantly reduced.[101] There was no significant change in peak oxygen consumption, minute ventilation over carbon dioxide slope, tricuspid annular plane systolic excursion, or RV fractional area change.[101] This study provides evidence that short-term administration of anakinra is safe with a potential reduction in symptom burden. A larger and longer duration trial will be needed to determine the utility of anakinra for PAH treatment.

Anastrozole

Targeting the estrogen pathway in PAH is rooted in the observation that there is a consistent and substantial (≈3–4:1) female predominance in the incidence of PAH.[102–105] Moreover, estrogen is linked to reduced BMPR2 expression[57] and metabolic derangements[106] in the pulmonary vasculature. Anastrozole is an antiestrogen compound that inhibits aromatase, an enzyme that catalyzes the formation of estradiol from testosterone.[107] Anastrozole is currently used as an adjuvant in postmenopausal women with hormone receptor–positive breast cancer.[108] Anastrozole is well tolerated in breast cancer patients, with common side effects including gastrointestinal discomfort, hot flashes, and gynecological disturbances. Long-term use of anastrozole can reduce bone mineral density.[109]

The beneficial effects of anastrozole are observed only in female rodents in preclinical PAH models. For example, female mice exposed to chronic hypoxia (10% O_2 for 14 days) and then treated with anastrozole (0.3 or 3 mg/kg per day) for 14 additional days at hypoxia have reduced adverse remodeling of pulmonary arteries, lower RV systolic pressure (RVSP), and less RVH.[57] In contrast, hypoxic male mice experience no benefit with anastrozole treatment.[57] In a regression protocol of SU-5416 hypoxia rats, anastrozole (0.03, 0.3, or 3 mg/kg per day for 14 days during normoxia) decreases the number of occluded and remodeled pulmonary arterioles but, again, only in female rats.[57] The sex-specific effects may be due to differences in aromatase levels in PASMCs. Specifically, male mice, rats, and humans have less aromatase in PASMCs than their female counterparts.[57] Intriguingly, in the hypoxic mice and

SU-5416 hypoxia rat experiments described earlier, anastrozole increases PASMC BMPR2 expression (Figure 4) but only in cells derived from females.[57] However, other mechanisms may also exist because the beneficial effects of anastrozole are also observed in inducible BMPR2 R899X transgenic mice. In this study (conducted exclusively in female mice), anastrozole (0.3 mg/kg per day) was used in combination with fulvestrant, a selective estrogen receptor degrader,[110] to more fully inhibit estrogen signaling. Anastrozole and fulvestrant increase lung expression of PPAR-γ (peroxisome proliferator-activator γ) and CD36 (which regulates fatty acid uptake and insulin sensitivity[111]) and improve insulin sensitivity (Figure 5).[106] Conversely, estrogen reduces insulin-induced membrane mobilization of GLUT4 (glucose transporter type 4) in pulmonary microvascular endothelial cells, which may underlie the negative effects of estrogen on insulin sensitivity.[106] Anastrozole and fulvestrant reduce the percentage of muscularized pulmonary arteries and lower RVSP.[106] Thus, anastrozole's sex-specific efficacy may relate to beneficial effects of estrogen inhibition on BMPR2 signaling and/or altered metabolism.

In a clinical trial of 18 male and female PAH patients, randomized in a 2:1 fashion to anastrozole (1 mg/day) or placebo for 3 months, anastrozole significantly decreased 17β-estradiol levels and increased 6MWD by a median distance of 26 m versus placebo.[112] However, there was no improvement in RV function and quality of life, and this trial did not assess invasive hemodynamics. These initial pilot data have led to the PHANTOM (Pulmonary Hypertension and Anastrozole) trial (ClinicalTrials.gov identifier NCT03229499). PHANTOM is a multicenter, double-blind, randomized, placebo-controlled trial that will investigate whether anastrozole (1 mg/day) for 1 year alters outcomes in 84 NYHA functional class I to III PAH patients on stable PAH-specific therapy. Change in 6MWD will be the primary end point, with secondary end points including changes in RV function, NT-pro-BNP, biomarkers of anastrozole treatment, symptomatic burden, daily activity, time to clinical worsening, and adverse side effects. The results of PHANTOM will help determine the efficacy of anastrozole in PAH.

The impact of estrogen inhibition with tamoxifen, a selective estrogen receptor blocker,[113] is also being investigated in PAH. A single-center, double-blind, randomized, placebo-controlled trial will be conducted at Vanderbilt University. The effects of tamoxifen (20 mg 3 times/day for 24 weeks) will be examined in 24 PAH patients (ClinicalTrials.gov identifier NCT03528902). The inclusion criteria for this trial include patients who have idiopathic, heritable, or drug- or toxin-induced PAH or PAH associated with connective tissue disease and who are classified as World Health Organization (WHO) functional class I to III and able to walk 150 to 550 m during a 6MWD test. Important exclusion criteria include treatment with any therapy that modulates sex hormones, pregnancy, WHO functional class

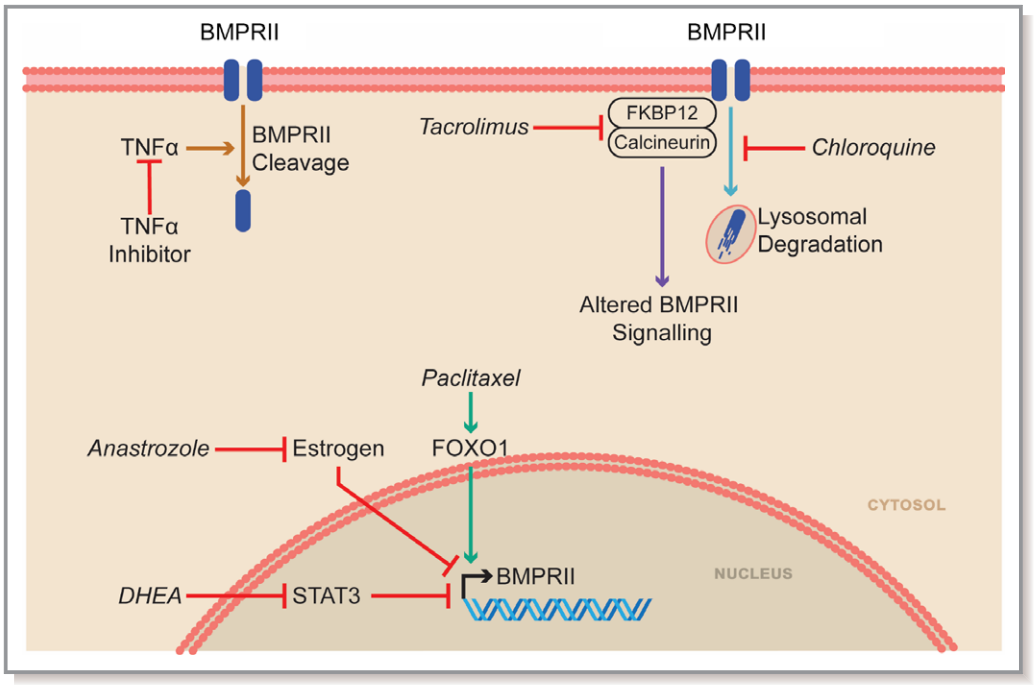

Figure 4. Medications that can augment the BMPR2 pathway as a therapeutic strategy for PAH. BMPR2 indicates bone morphogenic protein receptor 2; DHEA, dehydroepiandrosterone; FKBP12, 12-kDa FK506-binding protein; FOXO1, forkhead box protein O1; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α.

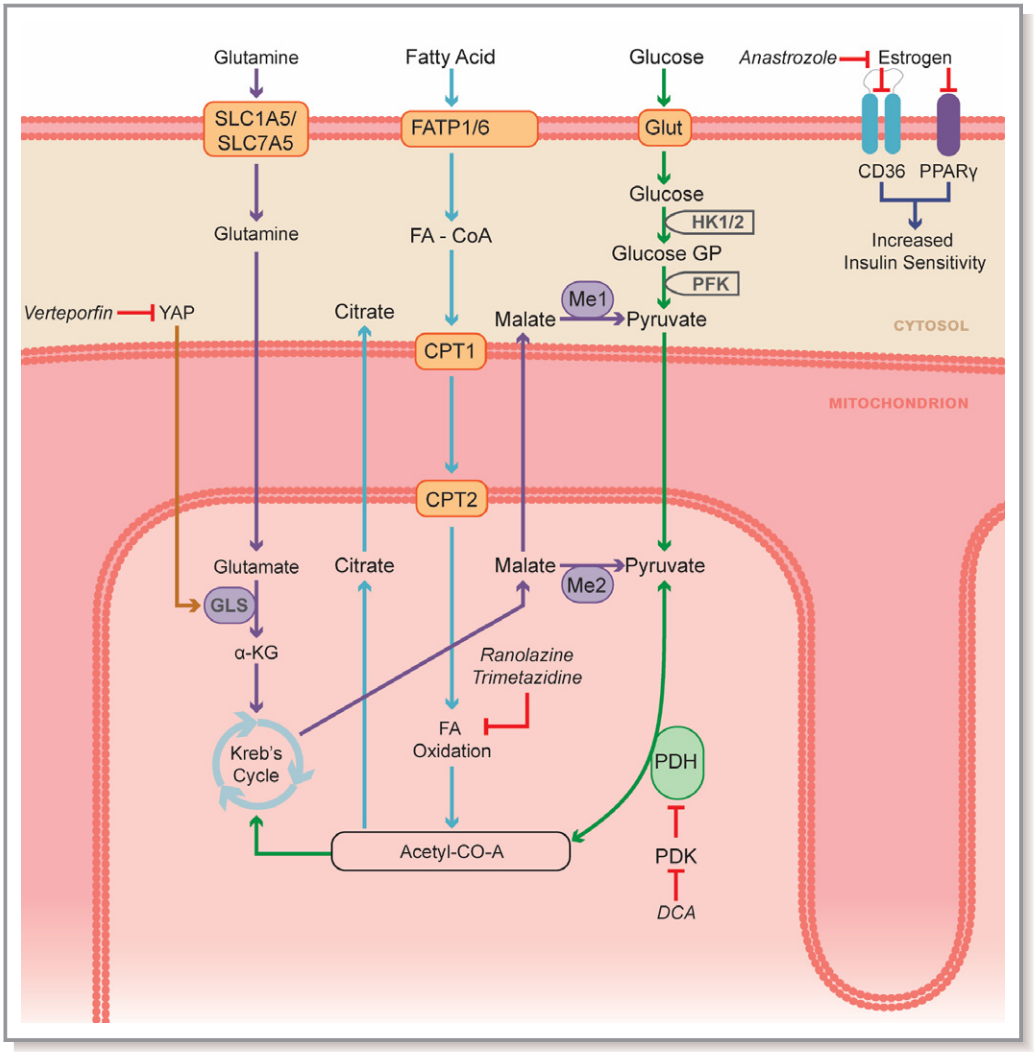

Figure 5. Pathological metabolic changes can be targeted with several available medications. α-KG, α-ketoglutarate; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; DCA, dichloroacetate; FA, fatty acid; FATP, fatty acid transport protein; GLS, glutaminase; Glut, glucose transporter; HK, hexokinase; Me, malic enzyme; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PPAR-γ, peroxisome proliferator-activator-γ; SLC, solute carrier family; YAP, Yes-associated protein.

IV, initiation of any PAH therapy within 3 months before visit, and use of either bosentan or selexipag, as these medications may have drug–drug interactions with tamoxifen.

Although estrogen inhibition holds promise for PAH treatment, we need to carefully observe how it will affect RV function. Evidence shows that estrogen augments RV function as 17β-estradiol supplementation increases exercise capacity in both male and female SU-5416 hypoxia rats, likely via an anti-inflammatory effect and inhibition of RV cardiomyocyte apoptosis.[114] Furthermore, 17β-estradiol administration to SU-5416 hypoxia female rats increases expression of PGC-1α (peroxisome proliferator–activated receptor γ coactivator 1α) and maintains mitochondrial mass and oxidative capacity in the right ventricle.[115]

Apabetalone

The balance of PASMC proliferation and apoptosis is an attractive target for PAH therapy because the imbalance of these 2 processes is repeatedly observed in PAH.[116] Increased expression of the epigenetic regulator BRD-4 (bromodomain-containing protein 4) is observed in lung extracts, distal pulmonary arteries, and isolated PASMCs of PAH patients.[117] The upregulation of BRD-4 depends on the downregulation of miR-204, a microRNA that represses

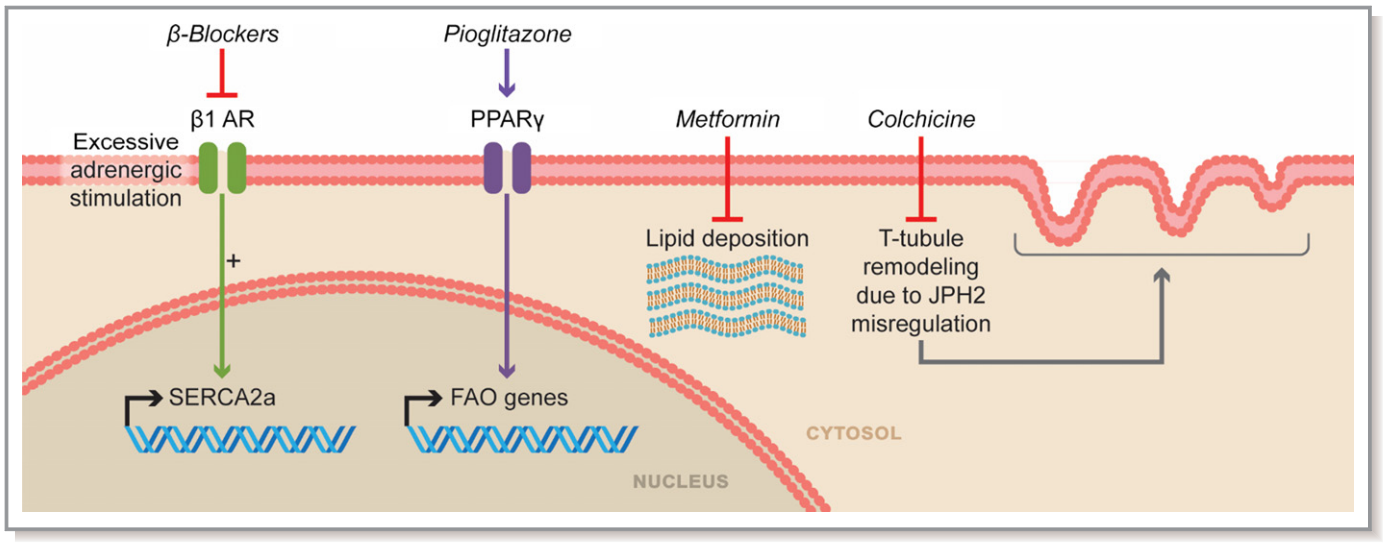

Figure 6. Pathologic pathways in the right ventricle that can be inhibited to improve right ventricular function. β1 AR indicates β1 adrenergic receptor; FAO, fatty acid oxidation; JPH2, junctophilin 2; PPAR-γ, peroxisome proliferator-activator-γ; SERCA2a, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

BRD-4.[117] BRD-4 regulates transcription of many genes through its interaction with acetylated histones. Increased abundance of BRD-4 promotes cell survival and inhibits apoptosis.[117] Apabetalone is a BRD-4 inhibitor that is currently being evaluated in patients with coronary artery disease,[118] although it is not yet approved for clinical use. In short-term clinical trials ranging from 3 to 6 months, apabetalone is well tolerated, but evidence suggests it may cause mild transaminase elevation.[118]

The beneficial effects of BRD-4 inhibition in PAH is observed in human PASMCs and in SU-5416 hypoxia rats. In cultured PASMCs, BRD-4 inhibition with either small interfering RNA or JQ1, a nonspecific BRD protein inhibitor, reverses upregulation of oncogenic proteins, such as NFATC2 (nuclear factor of activated T cells 2), Bcl-2 (B cell lymphoma 2), and survivin while simultaneously increasing expression of p21, an inhibitory cell-cycle regulator (Figure 2).[117] These molecular changes are accompanied by a reduction in proliferation and heightened apoptosis in cultured PASMCs.[117] In SU-5416 hypoxia rats (3 weeks of 10% O_2, then return to normoxia), treatment with nebulized JQ1 (1 μmol every 4 days for 2 weeks at 5 weeks after SU-5416 injection) prevents proliferation and promotes apoptosis of PASMCs, in turn reducing mPAP and increasing cardiac output.[117]

Apabetalone is currently being investigated in a single-arm trial in a 2-center study. Ten PAH patients who are WHO functional class II or III and on stable PAH therapy for >4 months will receive 100 mg of apabetalone twice a day for 16 weeks (ClinicalTrials.gov identifier NCT03655704). The primary end point in this trial is change in PVR. Secondary end points will include change in mPAP, right atrial pressure, mixed venous saturation, 6MWD, WHO functional class, NT-proBNP, quality of life, and change in biomarkers of vascular calcification, inflammation, complement, acute phase response, fibrogenesis, and metabolism.

β-Adrenergic Blockers

Biological plausibility for application of β-adrenergic blockers in PAH is supported by the observation that there is extreme neurohormonal activation in RV failure secondary to PAH (Figure 6).[119] Indeed, the degree of autonomic activation, both systemically and in the RV, is greater in PAH than in left ventricular (LV) failure syndromes.[119,120] β-Adrenergic blockers antagonize β-adrenergic receptors and thus could combat the effects of the marked systemic activation of the sympathetic nervous system that characterizes PAH.[119,120]

β-Adrenergic blockers are well tolerated if not administered to patients in decompensated heart failure, but common adverse effects include fatigue, bradycardia, and hypotension.[121] Clinically, β-adrenergic blockers have several indications including treatment of systemic hypertension,[121] arrhythmias,[121] angina pectoris,[121] prophylaxis for variceal bleeding in select cirrhotic patients,[122] and left-sided systolic heart failure.[123] In fact, β-adrenergic blockers are one of the main treatment strategies in LV systolic dysfunction because chronic β-adrenergic blocker therapy causes beneficial LV reverse remodeling and can increase LV ejection fraction[124] while improving survival.[123,125–127]

Preclinical studies demonstrate that antagonism of neurohormonal activation by chronic β-adrenergic receptor blockade or inhibition of GRK2 (G-protein-coupled receptor kinase 2)–mediated β-adrenergic receptor uncoupling improves RV

function, reverses RV remodeling, and restores RV β-adrenergic receptor signaling pathways.[128–132] Arotinolol (an α- and β-adrenergic receptor blocker) at a dose of 0.25 mg/kg per day starting at the time of monocrotaline injection prevents development of PAH and RVH.[130] Bisoprolol (a β1-cardioselective blocker) at a dose of 10 mg/kg per day starting 10 days after monocrotaline injection decreases RV fibrosis and inflammation, restores RV β-adrenergic signaling, and improves RV function.[129] Carvedilol (a nonselective α1/β1/β2-adrenergic receptor antagonist) at a dose of 15 mg/kg per day has beneficial effects on the right ventricle in both the SU-5416 hypoxia and MCT models.[128] In SU-5416 hypoxia rats, carvedilol, initiated at return to normoxia and maintained for 4 weeks, increases SERCA2a (sarco/endoplasmic reticulum Ca^{2+}-ATPase) mRNA levels (Figure 6), reduces RVH, improves RV function, reverses RV remodeling, and augments exercise capacity.[128] In MCT rats, carvedilol given 2 weeks after monocrotaline injection also enhances RV function, blunts RVH, and improves survival.[128]

Although considerable evidence shows that β-adrenergic blockers have a favorable effect on the right ventricle, substantial data indicate that β-adrenergic blockers may also hasten pulmonary vascular remodeling. For instance, arotinolol reduces pulmonary arterial pressures in MCT rats.[130] Furthermore, nebivolol increases nitric oxide activity, whereas carvedilol promotes vasodilation through α-adrenergic receptor antagonism.[133] Finally, propranolol blocks protein kinase C activity which, as a result, promotes nitric oxide synthesis.[134] These agents may have favorable effects on both the pulmonary vasculature and the right ventricle in PAH.

Several retrospective human studies show chronic β-adrenergic blocker therapy is safe when given at very low doses and slowly uptitrated in PAH patients who are not in overt heart failure. Under these circumstances, use of multiple β-adrenergic blockers, including metoprolol, atenolol, carvedilol, propranolol, nadolol, and labetalol, is not associated with increased mortality in PAH patients.[135–137] Based on the preclinical data and retrospective safety data in patients with PAH, several small clinical trials have evaluated the safety and efficacy of β-adrenergic blockers in PAH. In an open-label study of 6 PAH patients, treatment with carvedilol (median dose of 18.75 mg twice daily) was well tolerated and significantly improved RV ejection fraction (RVEF), as measured by cardiac MRI.[138] Treatment with bisoprolol (up to 10 mg/day for 6 months) in a randomized, double-blind, placebo-controlled, cross-over study of 19 PAH patients was also well tolerated. However, bisoprolol reduced cardiac index and exercise capacity and failed to improve RVEF.[139] More recently, in a randomized, double-blind, placebo-controlled trial of 20 PAH patients, use of carvedilol improved several surrogate end points.[140] Carvedilol treatment reduced glucose uptake in the right ventricle, as quantified by positron emission tomography, and increased β-adrenergic receptor density in circulating white blood cells. Carvedilol treatment significantly improved RV function (increased fractional area change on echocardiography) at 3 months but not at 6 months of follow-up.[140] However, carvedilol treatment did not increase exercise capacity or cardiac output.[140] Carvedilol was well tolerated, with no serious adverse events in participants receiving a fixed low dose (3.125 mg twice a day, n=10) or doses up to 25 mg twice a day (n=4).

Clearly, larger and longer studies are required to definitively examine the safety and efficacy of β-adrenergic blockers for improving RV function in PAH. Future studies should consider carvedilol over bisoprolol because it offers potentially beneficial vasodilator and antioxidant pleiotropic effects.[141,142] Furthermore, carvedilol, acting as a "biased" ligand, stimulates β-arrestin signaling and antagonizes G-protein–mediated signaling.[143] This is important because β-arrestin–dependent signaling is cardioprotective in the presence of chronic catecholamine stimulation,[144] which may explain why carvedilol reduces mortality by 50% in animal studies.[128] In conclusion, β-blockers should be tested mainly in PAH patients with reduced RV function because these patients are more likely to have increased neurohormonal activation[119] and thus have a higher likelihood of receiving a beneficial effect.

Chloroquine

As discussed, increasing BMPR2 signaling is a promising mechanism for PAH treatment. In cultured PASMCs, chloroquine increases BMPR2 protein levels by modulating autophagy (Figure 4).[58] Chloroquine was initially used to treat malaria; however, its uses have expanded to include treatment of rheumatologic disease such as rheumatoid arthritis and systemic lupus erythematosus.[145] Chloroquine is well tolerated in long-term use, although screening is needed for ocular side effects, especially in patients with impaired renal function.[145]

In cultured PASMCs, chloroquine increases BMPR2 protein levels, slows proliferation, and promotes apoptosis.[58] In vivo, chloroquine (20 or 50 mg/kg per day) given to rats at the time of monocrotaline injection attenuates the severity of PAH, as demonstrated by reduction in pulmonary vascular obstruction, RVSP, and RVH.[58] When given after PAH is established (3 weeks after monocrotaline injection), chloroquine (50 mg/kg per day) reduces the number of muscularized pulmonary arteries, RVSP, and RVH.[58] The scientific rigor score supporting the use of chloroquine is 2 (Table 1), but chloroquine's long and extensive track record of safe use in humans with numerous diseases (including inflammatory conditions relevant to PAH) suggest that it could be considered for PAH patients. However, the doses used in preclinical

studies may not be achievable in humans (Table 2), and dose ranging studies will be required. There are currently no trials to examine the use of chloroquine in PAH.

Colchicine

Drugs that target both the pulmonary vasculature and the right ventricle have great potential for PAH. Colchicine, an anti-inflammatory medication that is used to treat flares of the crystalline arthropathy gout,[146] familial Mediterranean fever,[147] and chronic or relapsing pericarditis,[148] has potential to reduce PASMC proliferation via anti-inflammatory mechanisms[149] and to improve RV function by combating t-tubule derangements.[67] When used clinically, colchicine has several side effects, with the most prominent being dose-dependent gastrointestinal disturbances (nausea, vomiting, and diarrhea).[150] However, at a dose of 0.5 mg twice a day, colchicine was safely tolerated for 6 months in a clinical trial of LV systolic heart failure patients.[151]

Administration of colchicine (1.0 mg/kg/day) for 5 days beginning 5 days after monocrotaline administration significantly reduces levels of TNF-α (tumor necrosis factor α), NF-κb (nuclear factor κB), MMP9, and TGF-β (transforming growth factor β) in both the lungs and the right ventricle. The improved inflammatory mediator profile achieved by colchicine is associated with diminished severity of pulmonary hypertension (Figure 3).[149]

In RV cardiomyocytes, colchicine counteracts the pathologically remodeled microtubule cytoskeleton and corrects the detrimental JPH2 downregulation that contributes to RV dysfunction in PAH. JPH2, a protein that is essential for proper t-tubule structure and function,[152] is decreased in monocrotaline RV cardiomyocytes and this leads to disrupted t-tubule structure.[67] The structural changes in the t-tubules ultimately contribute to RV hypokinesis (Figure 6).[67] In MCT rats, colchicine administration (0.5 mg/kg 3 times/week) starting 1 week after monocrotaline injection increases JPH2 protein levels by depolymerizing the hyperstabilized microtubule cytoskeleton, which slows pathological t-tubule remodeling. These molecular and cellular changes improve RV function, increase cardiac output, and enhance exercise capacity.[67] Importantly, although colchicine directly augments RV function, as shown by a disruption of the strong relationship between elevated pulmonary arterial pressures and RV dysfunction, it also regresses pulmonary vascular disease burden.[67]

Because colchicine has the potential to target pathological processes in both the pulmonary vasculature and the right ventricle, it could be investigated in PAH patients. Barriers leading to a clinical trial include the scientific rigor score of only 3 (Table 1), and the human doses equivalent to those used in animal studies may be difficult to achieve (Table 2).

Dehydroepiandrosterone

Dehydroepiandrosterone, or DHEA, is a naturally occurring, cholesterol-derived steroid hormone that is a precursor for both estrogen and testosterone.[153] DHEA has been used to treat aspects of adrenal insufficiency and postmenopausal changes including sexual dysfunction and symptoms of menopause.[154,155] Although largely shown to be ineffective in clinical trials for these indications, the safety profile is excellent, with no major adverse effects in a meta-analysis of 1188 women taking DHEA, but there are reports of hair growth and acne.[155,156] DHEA has multiple biological actions in vivo including modulation of endothelial function, anti-inflammatory effects, increasing insulin sensitivity, and neuroprotection.[153] In PAH, DHEA promotes pulmonary vasodilation through reduction of intracellular calcium,[157] normalizes the PASMC proliferation/apoptosis balance,[158] and potentially improves RV function by mitigating oxidative stress.[159] Clinically, lower serum levels of DHEA are associated with an increased risk of developing PAH in men.[160] Furthermore, in men with PAH, higher serum levels of DHEA are associated with lower right atrial pressure and PVR.[160]

Several independent publications demonstrate that DHEA is efficacious in multiple preclinical models of PAH. In rats with chronic hypoxia (0.5 atm of pressure), treatment with DHEA (30 mg/kg every other day) can prevent and reverse pulmonary hypertension.[157] DHEA reduces levels of intracellular calcium via increased expression and activity of the calcium-activated potassium channel in PASMCs.[157] In MCT rats, DHEA (10 mg/kg daily starting 18 days after monocrotaline) significantly reduces pulmonary vascular remodeling, mPAP, and RVH while normalizing treadmill walk distance.[158] At the molecular level, DHEA decreases STAT3 (signal transducer and activator of transcription 3) activation, which restores the apoptosis/proliferation balance by reducing NFATc2 and survivin levels (Figure 2) and increasing BMPR2 levels (Figure 4).[158] Finally, in SU-5416 hypoxia rats (10% O_2 for 3 weeks), treatment with DHEA (1% DHEA-containing food) starting at return to normoxia for 5 weeks significantly blunts pulmonary vascular remodeling and RVH, improves RV function, and augments cardiac index.[159] In the right ventricle, DHEA treatment lessens collagen expression and the number of cardiomyocytes undergoing apoptosis.[159] Moreover, DHEA moderates oxidative stress, as demonstrated by a reduction in NADPH oxidase subunit $gp91^{phox}$ (Figure 2) and tissue NADPH levels.[159] Thus, substantial evidence suggests that DHEA may affect multiple pathological processes in PAH.

DHEA is currently being investigated in the EDIPHY (Effects of DHEA in Pulmonary Hypertension) trial. EDIPHY is a crossover trial that will be conducted in 24 PAH patients who are on stable PAH-directed therapy for at least 12 weeks (ClinicalTrials.gov identifier NCT03648385). Patients will be

randomized to placebo or DHEA (50 mg daily) for 18 weeks, there will be a 4-week wash-out period, and then patients will be treated on the other arm of the trial. The primary outcome is change in RV longitudinal strain, as determined by cardiac MRI. Secondary outcomes will include RVEF, NT-pro-BNP, sex hormone levels, 6MWD, WHO functional class, symptom burden, and adverse effects.

Dichloroacetate

In PAH, PDK (pyruvate dehydrogenase kinase), a family of enzymes that negatively regulate glucose oxidation,[66,161] is activated through numerous mechanisms, including epigenetic activation of HIF-1α.[8] Activated PDK phosphorylates and inhibits mitochondrial PDH (pyruvate dehydrogenase), which suppresses oxidative metabolism, leading to a metabolic shift that favors uncoupled glycolysis (the Warburg phenomenon).[66] In PAH, the Warburg effect, caused in part by PDK upregulation, occurs in both the pulmonary vasculature and the right ventricle (Figure 5).[66] Warburg metabolism retains energetic stability (achieved by high rates of glycolytic flux) while minimizing basal rates of unstimulated apoptosis, which promotes cellular proliferation (in PASMCs, endothelial cells, and vascular fibroblasts) and hypertrophy (in RV cardiomyocytes).[13]

Dichloroacetate is a PDK inhibitor that has long been used to treat patients, particularly children with inherited mitochondrial disorders.[162] The most common side effect of dichloroacetate is neuropathy, which is usually reversible with drug discontinuation.[161] An extensive series of experiments shows dichloroacetate can prevent and regress PAH in multiple rodent models. Dichloroacetate's beneficial effects on the pulmonary vasculature and the right ventricle are achieved through several mechanisms. Dichloroacetate restores the hypoxia-dependent whole-cell potassium current in PASMCs.[64] When given to chronically hypoxic rats (10% O_2), dichloroacetate (70 mg/kg per day) partially reverses downregulation of voltage-gated potassium channels, such as Kv1.5 and Kv2.1, in PASMCs. By retaining voltage-gated potassium channel expression and function, dichloroacetate maintains the membrane potential of the PASMCs and thereby prevents activation of the large conductance voltage-gated calcium channel. This reduces pulmonary vasoconstriction and results in less severe pulmonary vascular remodeling, lower pulmonary arterial pressures, and higher cardiac output in protocols for both prevention (dichloroacetate given day 1 of hypoxia) and regression (dichloroacetate initiated on day 10 of hypoxia).[64] In MCT rats, dichloroacetate (80 mg/kg per day) increases expression of potassium channel Kv1.5 in lung extracts.[12] This promotes vasodilation and apoptosis of PASMCs by normalization of potassium current regulation and membrane potential.[12] Furthermore, dichloroacetate depolarizes mitochondria, which restores physiological H_2O_2 production and induces PASMC apoptosis.[12] In a regression model, dichloroacetate reverses pulmonary vascular remodeling, leading to lower pulmonary pressures, normalization of RV thickness, and reduced mortality.[12]

In addition to the effects on the pulmonary vasculature, dichloroacetate (70 mg/kg per day) augments RV function via metabolic reprogramming, leading to greater glucose oxidation, which improves RV function in both PA-banded (treatment starting day of PA banding) and MCT rats (10 days after monocrotaline).[163] Also, dichloroacetate restores expression of potassium channels Kv1.2, Kv1.5, and Kv4.2 in the right ventricle, which normalizes cardiac repolarization—evident from a shortening of the QT interval on the ECG.[163] The beneficial effects of dichloroacetate are more pronounced in MCT rats than in PA-banded rats, suggesting that correction of the pulmonary vasculature plays a major role in beneficial effects of this drug on the right ventricle; importantly, however, there is a direct effect on RV function in isolated heart assessments.[163] In fact, dichloroacetate (1 mM for 40 minutes) acutely increases monocrotaline RV contractility in the working heart model.[163] Finally, in the fawn-hooded rat model of PAH, dichloroacetate (0.75 g/L of drinking water for 6 months) decreases activation of FOXO1 (forkhead box protein O1), a transcription factor that upregulates RV PDK4 expression. The reduction in FOXO1 diminishes PDK4 levels and restores PDH activity, which improves glucose oxidation and contractile function in RV cardiomyocytes.[164] In summary, dichloroacetate consistently reduces RVH and increases cardiac output and exercise capacity in multiple preclinical models of PAH.

The safety and efficacy of dichloroacetate were examined in ex vivo human PAH lungs and in PAH patients. In an ex vivo human lung perfusion system, dichloroacetate stimulates PDH activity and improves oxygen consumption in PAH lungs.[165] However, the positive effects are not observed in all PAH patients' lungs. In fact, the beneficial effects of dichloroacetate depend on the absence of polymorphisms in SIRT3 (sirtuin 3) or UCP2 (uncoupling protein 2), 2 proteins that regulate mitochondrial function in a PDK-independent manner.[166–168] In a 4-month open-label trial in 20 PAH patients, dichloroacetate reduced PVR and increased 6MWD. However, the greatest effects on PVR reduction and 6MWD changes were observed in patients with normal or low polymorphism scores or those with normal predicted function of *SIRT3* and *UCP2*.[165] Dichloroacetate doses up to 6.25 mg twice daily were well tolerated, but doses of 12.5 mg twice daily were associated with significant (but reversible) peripheral neuropathy.[165] In summary, these data demonstrate that PAH patients, who are genetically susceptible to metabolic targeting with dichloroacetate, experience improvements in hemodynamics and exercise capacity. This trial lays the

foundation for a personalized medicine approach using dichloroacetate in PAH patients who lack significant polymorphisms in the *SIRT3* and *UCP2* genes.

Metformin

Therapies that can target both the right ventricle and the pulmonary vasculature would be optimal for PAH treatment. Metformin, a biguanide that is frequently used to treat type 2 diabetes mellitus,[169] has potential to promote pulmonary vasodilation by increasing endothelial nitric oxide synthase,[170] to mitigate PASMC proliferation by inhibiting the pro-proliferative MAPK (mitogen-activated protein kinase),[170] to negate the estrogen pathway via inhibition of aromatase transcription,[171] and to combat pathological lipid deposition in the right ventricle.[172] Clinically, metformin has a long safety history, but the most common side effect is gastrointestinal discomfort. A potentially dangerous adverse effect, lactic acidosis, can occur, particularly in patients with renal impairment.[173] However, the reported incidence of metformin-induced lactic acidosis is <10 per 100 000 patient-years.[173]

In preclinical models, the beneficial effects of metformin are mediated through multiple molecular mechanisms. In hypoxic rats (inhaled oxygen tension 380 mm Hg for 21 days), metformin (100 mg/kg per day starting day 1 or 14 of hypoxia) prevents or slows progression of pulmonary hypertension. Metformin increases the amount of phosphorylated endothelial nitric oxide synthase in PA extracts and decreases Rho kinase activity, thereby reducing PASMC contractility.[170] Moreover, metformin inhibits MAPK activity, which slows PASMC proliferation[170] (Figure 2). In MCT rats (treatment starting at day of monocrotaline injection), metformin (100 mg/kg per day) curtails adverse pulmonary vascular remodeling, decreasing pulmonary pressures and RVH.[170] In female SU-5416 hypoxia rats (hypobaric: 412 mm Hg for 2 weeks, then return to room pressure), metformin (100 mg/kg per day) starting 3 weeks after return to room air pressure inhibits aromatase transcription, which subsequently lowers both lung and circulating estrogen levels.[171] Moreover, metformin augments PASMC AMP-kinase activity, which inhibits PASMC proliferation (Figure 2), slows pulmonary vascular remodeling, lowers pulmonary arterial pressures, and attenuates RVH.[171] Finally, metformin also has potential to benefit the right ventricle directly by decreasing pathologic lipid deposition (Figure 6). In BMPR2 R899X transgenic mice, metformin (25 g/kg of high fat chow starting at 6 weeks of age and continuing for 6 weeks) reduces lipid deposition in the right ventricle.[172] However, metformin does not significantly improve systolic or diastolic measures of RV function.[172]

Metformin use in PAH patients is currently being investigated in a phase 2 clinical trial (ClinicalTrials.gov identifier NCT01884051). The primary end points are measures of insulin resistance, urinary and plasma oxidant stress markers, RV lipid content and oxidative metabolism, and drug safety. Secondary end points will include lung metabolism, as quantified by 18F-fluorodeoxyglucose uptake, BMPR2 expression in peripheral blood mononuclear cells, glucose and lipid metabolites, RVEF and RV volumes using MRI, insulin sensitivity indexes, and 6MWD.

Olaparib

Emerging evidence demonstrates that DNA damage is more abundant in PAH PASMCs than in healthy PASMCs.[174] However, PAH PASMCs are able to proliferate despite accumulation of harmful DNA damage. The DNA repair enzyme PARP1 (poly[ADP-ribose] polymerase 1) expression is increased in PASMCs from PAH patients,[174] which may explain the paradox of proliferation despite compromised DNA integrity. Interestingly, markers of DNA damage and elevated PARP1 expression can be recapitulated in healthy PASMCs via incubation with TNF-α, showing that inflammation promotes DNA damage in PAH.[174] In PAH PASMCs, PARP1 antagonism reduces proliferation and promotes apoptosis (Figure 2).[174] Olaparib is a PARP1 inhibitor that is currently approved for treatment of ovarian cancer associated with breast cancer susceptibility genes (*BRCA1* [BRCA1, DNA repair associated] or *BRCA2* [BRCA2, DNA repair associated]) and BRCA/HER2 (human epidermal growth factor receptor 2)–negative metastatic breast cancer.[175] Importantly, olaparib improves progression-free survival in BRCA mutation carriers with HER2-negative metastatic breast cancer.[176] Olaparib has substantial adverse effects including bone marrow suppression, abdominal pain, and nausea/vomiting.[175]

In PAH PASMCs, PARP1 inhibition normalizes miR-204, which in-turn decreases NFATc2 and HIF-1α levels.[174] Furthermore, PARP1 suppression reduces intracellular calcium concentration and normalizes mitochondrial membrane potential of PAH PASMCs.[174] In whole-animal experiments, olaparib reduces PAH severity in MCT and SU-5416 hypoxia (10% O_2 for 3 weeks) rats in regression approaches. Olaparib (6 mg/kg per day for 2 weeks) given either 14 days after monocrotaline or 5 weeks after SU-5416 inhibits PASMC proliferation and increases PASMC apoptosis.[174] This results in lower mPAP and less RVH in both MCT and SU-5416 hypoxia rats.[174]

Olaparib is currently being investigated in a phase 1 open-label clinical trial (ClinicalTrials.gov identifier NCT03251872). Six PAH patients who are WHO functional class II or III and on stable PAH therapy for at least 4 months will be treated with olaparib (400 mg twice daily) for 16 weeks, with the primary end point being change in PVR. Secondary end points will include change in invasive hemodynamics, RV function, volume, and mass quantified by cardiac MRI, WHO functional class, NT-proBNP levels, and quality of life.

Paclitaxel

FOXO1 is one of the many proteins that regulate PASMC proliferation through its ability to induce apoptosis.[177] In PAH patients and in MCT and SU-5416 hypoxia rats, total FOXO1 protein levels are diminished and phosphorylated levels of FOXO1 are elevated in the pulmonary vasculature.[177] The change in ratio of total FOXO1/phosphorylated FOXO1 is associated with development of pulmonary hypertension via promotion of PASMC proliferation.[177]

Paclitaxel, a microtubule stabilizer that is used as a chemotherapeutic agent for multiple types of cancer,[178] has the ability to increase FOXO1 and inhibit phosphorylation of FOXO1.[177] Paclitaxel, like many chemotherapeutic agents, has several significant side effects including myelosuppression, nausea, diarrhea, and peripheral neuropathy, limiting its widespread use.[179] However, paclitaxel promotes nuclear localization and activation of FOXO1 in PASMCs. Activated FOXO1 slows cellular proliferation and induces apoptosis via alteration of expression of multiple cell-cycle regulators and promotion of BMPR2 signaling in PASMCs[177] (Figure 4). In disease regression studies of both MCT rats (5 mg/kg on days 21 and 28 after monocrotaline injection or aerosolized paclitaxel 1 mg/kg on days 21 and 28) and SU-5416 hypoxia rats (10% O_2 for 21 days and then paclitaxel 7 mg/kg on day 21 and 28), paclitaxel has significant therapeutic effects. Paclitaxel treatment activates BMPR2 signaling and induces PASMC apoptosis. These molecular and cellular changes result in reduction in RVSP, blunting of RVH and RV dilation, and improvement in RV function in both MCT and SU-5416 hypoxia rats.[177] In addition, paclitaxel may have beneficial effects via its ability to downregulate FOXM1 (forkhead box M1),[180] an oncogene that is also implicated in pathological pulmonary vascular remodeling.[181–183]

Paclitaxel use in PAH is not being investigated, likely due to significant side effects. However, use of an aerosolized form of paclitaxel could limit systemic effects, and because this approach is efficacious in preclinical models,[177] it may merit further investigation in patients. Paclitaxel has a scientific rigor score of 4 (Table 2), but the side effects suggest other therapies may take priority.

Ranolazine

The *Randle cycle* describes the reciprocal relationship between fatty acid oxidation (FAO) and glucose oxidation.[184] In RVH, the Randle cycle is observed and is associated with inefficient metabolism and impaired RV function.[185] Ranolazine is a partial inhibitor of FAO (Figure 5) and thus can combat the Randle cycle. Ranolazine is used to treat refractory angina pectoris in patients with coronary artery disease.[186] In addition to the FAO effects, ranolazine may block sodium and potassium channels.[186,187] Ranolazine is generally well tolerated but can result in prolongation of the QT interval.[186]

In pulmonary artery–banded rats, ranolazine was used to exploit the Randle cycle to enhance RV function by improving RV energetics.[185] Ranolazine (20 mg/day starting 3 weeks after pulmonary artery banding) reduces expression of Glut1, a glucose membrane transporter, and HK1 (hexokinase 1) and increases PDH activity,[185] compatible with its reduction of uncoupled glycolysis. Ranolazine promotes glycolytic oxidation and thereby suppresses FAO in the right ventricle. This metabolic shift reverses RVH, improves RV function, and enhances exercise capacity,[185] perhaps because glucose oxidation requires less oxygen per mole of ATP produced than does FAO. In MCT rats, ranolazine treatment (0.25 or 0.5% in chow starting 1 week after monocrotaline injection) regresses RVH and RV fibrosis, as demonstrated by histological assessment and a decrease in mRNA levels of collagen 1α1, CTGF, and TGF-β in the right ventricle.[188] Finally, ranolazine treatment renders isolated monocrotaline hearts less susceptible to stimulation-induced ventricular tachycardia and ventricular fibrillation, likely because of improvements in cardiac repolarization.[188]

Two small clinical trials tested the utility of ranolazine in PAH patients. First, the effects of a 500-mg dose at 6 hours (n=6 patients) and a 12-week extension phase (n=4 patients at a dose of 500 mg/day) were examined in a placebo-controlled trial. Acute administration of a single dose of 500 mg of ranolazine did not alter mPAP, PVR, or cardiac index; however, only 1 patient achieved the targeted therapeutic level of ranolazine at the 6-hour time point.[189] In the 12-week extension study of 4 patients (500 mg daily), cardiopulmonary exercise testing, 6MWD, NT-pro-BNP, symptom burden, and RV function as quantified by echocardiography did not change significantly.[189] Second, an open-label study using a higher dose of ranolazine (1000 mg twice/day) in 11 PAH patients with evidence of RV dysfunction on echocardiography (RV fractional area change <35% or tricuspid annular plane systolic excursion <1.6 cm) yielded more encouraging results. Three months of ranolazine treatment significantly improved functional class, RV size, and peak RV strain during exercise without significantly altering pulmonary vascular disease severity.[190]

There is an ongoing multicenter, double-blind, randomized (2:1 ranolazine:placebo) controlled trial that will examine whether 6 months of ranolazine (500–1000 mg twice a day) alters RV function. In this study, 24 patients with RV dysfunction (RVEF <45% on cardiac MRI) due to pulmonary hypertension caused by comorbidities other than left heart disease (ClinicalTrials.gov identifiers NCT01839110/NCT02829034) will be studied. The primary end point will be change in RVEF, as determined by cardiac MRI, with secondary end points to include 6MWD, functional class

assessment, 4-dimensional flow and T1 mapping from cardiac MRI, change in serum metabolites, microRNA changes in peripheral blood, quality of life as assessed by the 36-Item Short Form (SF-36) questionnaire, and adverse side effects.[191]

Rapamycin

As discussed earlier, mTORC can regulate PASMC proliferation. In addition, mTORC signaling can be directly inhibited by the mTOR inhibitor rapamycin. Rapamycin is used clinically as an immunosuppressing agent for solid organ transplant patients and is also used on drug-eluting coronary stents to prevent in-stent stenosis.[192] However, rapamycin has several side effects including immune suppression, thrombocytopenia, hyperlipidemia, and impaired wound healing.[192]

The utility of rapamycin in preclinical PAH is controversial because multiple publications have shown divergent results. In a rat model that combined pneumonectomy and monocrotaline (7 days after pneumonectomy), rapamycin (2.5 mg/kg per day) reduces pulmonary vascular remodeling and RVH when given in a preventative model (2 days before monocrotaline) but not in a reversal model (given 15 days after pneumonectomy).[193] In another prevention study, rapamycin (2 mg/kg/day) administration starting 1 day before monocrotaline injection slows pulmonary vascular remodeling resulting in blunted RVH.[194] The beneficial effects of rapamycin may be related to the ability of rapamycin to increase levels of HO-1 (hemoxygenase 1), a protein that promotes vasodilation.[194] In chronic hypoxic (0.5 atm of pressure) male and female mice, rapamycin (3 mg/kg per day starting 3 weeks after hypoxia) attenuates pulmonary vascular remodeling and subsequent RVH.[195] In a study of established PAH, treatment with rapamycin (2.5 mg/kg per day beginning 12 days after monocrotaline injection) reduced levels of phosphorylated S6 kinase in lung extracts, a downstream marker of the mTORC1 signaling. However, rapamycin treatment does not result in any differences in histological severity of pulmonary vascular remodeling, mPAP, cardiac index, or RVH.[196] These results suggest that rapamycin may not regress pulmonary hypertension despite S6 kinase inhibition. Finally, the effects of higher dose rapamycin (5 mg/kg per day) in both prevention and regression was investigated in MCT rats. In a prevention strategy (given the day of monocrotaline injection) rapamycin reduces pulmonary vascular remodeling, PA pressures, and RVH.[197] This is associated with an inhibition of S6 kinase signaling and Akt and GSK3 (glycogen synthase kinase 3) signaling, downstream effector kinases of the mTORC2 pathway (Figure 2).[197] When administered in a regression protocol (21 days after monocrotaline injection for 21 days), rapamycin reduces pulmonary vascular disease severity and blunts mTORC1 and mTORC2 signaling.[197]

Nab-rapamycin, an albumin-bound version of rapamycin that has been studied in nonhematologic malignancies,[198] is currently being studied in a phase 1 open-label clinical trial. In total, 25 PAH patients who are WHO functional class III or IV on 2 or more PAH-specific therapies with a 6MWD between 150 and 450 m will be treated with Nab-rapamycin (ClinicalTrials.gov identifier NCT02587325). The primary end point is the number of patients with an adverse effect. The dosing protocol is not currently outlined. It is difficult to translate the preclinical doses of rapamycin to Nab-rapamycin, so dosing studies may be needed.

Rituximab

PAH has a high prevalence of autoantibodies, particularly in the forms associated with scleroderma, and evidence of autoimmunity.[20] These data provide biological plausibility for the use of rituximab, an anti-CD20 antibody that targets B lymphocytes. CD20 is an antigen on the surface of B lymphocytes that gradually disappears as these cells mature into plasmocytes.[199] Rituximab was first used to treat non-Hodgkins B-cell lymphoma but is now used to treat several autoimmune diseases.[200] Rituximab is often used to treat conditions for which there is a clonal source of autoantibodies, such as rheumatoid arthritis and systemic lupus erythematosus.[200] Rituximab can be used for extended periods of time, but excessive immunosuppression can become a problem, and in some patients, reactivation of infections, such as hepatitis B, may occur.[201] Acute infusion of rituximab is often accompanied by infusion reaction, which includes fever, flushing, dyspnea, and chest pain. In fact, as many as 77% of patients experience a reaction during the first infusion.[202]

The therapeutic effects of an anti-CD20 antibody were examined in a novel model of PAH generated by ovalbumin immunization and administration of SU-5416. Ovalbumin immunization on days 1 and 7, combined with ovalbumin inhalation (1% ovalbumin in saline for 30 minutes twice a week for 4 weeks starting at day 14) and SU-5416 injection (20 mg/kg weekly starting on day 15), causes pulmonary hypertension. An anti-CD20 antibody (7 mg/kg on days 14, 17, and 21) reduces expression of HIF-1α, VEGF (vascular endothelial growth factor), and IL6 in the pulmonary vasculature (Figure 3). The change in the inflammatory milieu slows proliferation of PASMCs, which leads to lower mPAP and less RVH.[203]

Rituximab is currently being investigated in systemic sclerosis–associated PAH. In this multicenter, double-blind, randomized, placebo-controlled trial (ClinicalTrials.gov identifier NCT01086540), 60 patients with systemic sclerosis–associated PAH will be randomized to placebo or rituximab (2 infusions of 1000 mg 14-days apart) and followed for

48 weeks. The primary end point will be change in PVR at 24 weeks, with secondary end points including clinical worsening, carbon monoxide diffusing capacity, oxygen saturation, digital ulcers, severity of Raynaud phenomenon, 6MWD, biomarkers of disease progression, quality of life, and safety. An MRI substudy entitled Right Ventricular Response to Rituximab in Systemic Sclerosis–Associated Pulmonary Arterial Hypertension—A Magnetic Resonance Imaging Substudy, will quantify RV end-diastolic volume index and stroke volume, as determined by cardiac MRI, 24 weeks after drug treatment. This trial has completed enrollment, and the results should be available soon.

Rosiglitazone/Pioglitazone

Both preclinical and human studies have demonstrated PPAR-γ pathway dysregulation in PAH. For example, deletion of PPAR-γ in smooth muscle cells causes a form of PAH in mice.[204] Furthermore, in human PAH lungs, PPAR-γ mRNA is reduced.[205] Rosiglitazone and pioglitazone are thiazolidinediones that are PPAR-γ agonists used to treat type 2 diabetes mellitus,[206,207] and they show promising effects in preclinical PAH models.

In a novel PAH model of male apolipoprotein E knockout mice fed a high-fat diet, rosiglitazone treatment (10 mg/kg per day) improves insulin sensitivity, increases plasma adiponectin levels, and reverses pulmonary vascular remodeling, resulting in lower RVSP and less RVH.[208] At the molecular level, rosiglitazone treatment promotes adiponectin-mediated suppression of PDGF (platelet-derived growth factor)–induced PASMC proliferation[208] (Figure 2). In chronically hypoxic rats (12% O_2 for 4 weeks), rosiglitazone (8 mg/kg for 5 days/week) starting at day 1 of hypoxia increases PPAR-γ levels and decreases ET1 (endothelin 1) and VEGF levels, which slows pathologic pulmonary vascular remodeling and lowers PA pressures.[209] In hypoxic mice (10% O_2 for 3–5 weeks), rosiglitazone (10 mg/kg per day) treatment during the last 10 days of hypoxia has therapeutic effects. In hypoxic mice, rosiglitazone reduces PAH; the authors attribute this reduction to alteration of oxidative signaling (eg, reduced expression of Nox4 [NADPH oxidase 4]; Figure 1), decreased superoxide generation, blunted PDGF activation, and mitigation of PTEN (phosphatase and tensin homolog) downregulation in the lungs.[210]

In SU-5416 hypoxia rats (3 weeks of 10% O_2 followed by 6 weeks of normoxia), pioglitazone (20 mg/kg per day starting 1 week after return to normoxia) shows very promising results. Pioglitazone treatment nearly normalizes PA pressures via blunting of pulmonary vascular remodeling.[211] These changes manifest as improvements in RV function, as quantified by cardiac MRI and echocardiography. Moreover, RV glucose uptake and RV fibrosis are reduced.[211] At the organelle level, there is an improvement in mitochondrial structure and organization in RV cardiomyocytes. Furthermore, pioglitazone induces multiple genes in the FAO pathway (Figure 6), which improves utilization of fatty acids in neonatal rat cardiomyocytes. Finally, pioglitazone treatment reduces the levels of miR-197 and miR-146b in SU-5416 RV tissue; these 2 microRNAs are also upregulated in human PAH right ventricles.[211]

Although the preclinical data are promising and grade well with a scientific rigor score of 8 (Table 1), use of rosiglitazone and pioglitazone for PAH must be considered cautiously because both increase the risk of heart failure in diabetic patients.[212,213] Moreover, the preclinical doses are significantly higher than those maximally used in patients (Table 2). No ongoing trials are currently attempting to translate the positive preclinical results into human PAH patients.

Tacrolimus

BMPR2 signaling is suppressed in all forms of PAH, not just in familial PAH[60]; therefore, compounds that promote BMPR2 signaling may be efficacious in PAH. In a high-throughput assay that identified BMPR2 activators, tacrolimus, a calcineurin inhibitor used for immunosuppression in solid organ transplant patients,[214] was the strongest promoter of BMPR2 signaling out of 3756 compounds screened.[215] Although used frequently in solid organ transplant recipients, tacrolimus increases risk of infection, causes hypertension, and has a negative impact on renal function.[216]

Spiekerkoetter et al showed that tacrolimus stimulates BMPR2 signaling via calcineurin inhibition and sequestration of FK-binding protein 2 (12 kDa FK506-binding protein 2) (Figure 4) from the BMPR1 receptors ALK1 (activin receptor-like kinase), ALK2, and ALK3. This triggers signaling by SMAD1/5 (SMAD family members 1–5) and MAPK, leading to increases in ID1 (inhibitor of differentiation) transcription.[215] In human endothelial cells, tacrolimus induces expression of 2 downstream BMPR2 targets, ID1 and APLN (apelin), and improves endothelial function, as quantified by tube formation.[215] Tacrolimus (0.05 mg/kg per day) reverses pulmonary vascular remodeling, lowers RVSP, and reduces RVH in a mouse model of chronic hypoxia (10% O_2) superimposed on a BMPR2 endothelial cell knockout genotype.[215] Tacrolimus is also effective in combating established pulmonary hypertension in MCT rats (when administered 21 days after monocrotaline injection) and in SU-5416 hypoxia rats (10% O_2 for 3 weeks, normoxia for 5 weeks, and then treatment with tacrolimus for 3 weeks).[215]

Tacrolimus was tested in human PAH patients in compassionate use and in a randomized placebo-controlled trial. In 3 patients with end-stage PAH, addition of tacrolimus to conventional PAH therapies was examined. In all 3 patients,

tacrolimus treatment reduced symptomatic burden and improved exercise capacity.[217] In peripheral blood mononuclear cells, tacrolimus increases BMPR2 mRNA levels, which is associated with induction of downstream BMPR2 signaling molecules ID1, SMURF1 (SMAD-specific E3 ubiquitin ligase), and LIMK1 (Lim domain kinase 1).[217] In a phase 2a randomized controlled trial, the effects of 16 weeks of tacrolimus at 3 different target serum levels (<2, 2–3, and 3–5 ng/mL) were investigated in 14 PAH patients and compared with 6 placebo controls.[218] Overall, there was a heterogeneous response to tacrolimus regarding its ability to positively regulate mRNA levels of BMPR2 and ID1 in peripheral blood mononuclear cells.[218] Patients who increased BMPR2 to a greater extent tended to have greater improvements in 6MWD and RV function (as quantified by RV fractional area change and global longitudinal strain).[218] However, there were no significant differences in 6MWD and RV function when the pooled tacrolimus group was compared with the placebo group.[218] The widespread utility of tacrolimus in PAH is unproved, and perhaps better biomarkers are needed to help identify patients who may respond to tacrolimus.

Tocilizumab

The IL6 pathway is another potential target for PAH treatment because animal models have implicated a role for IL6 in PAH. IL6 knockout mice exhibit a blunted response to hypoxia-induced (10% O_2) pulmonary vascular remodeling,[219] whereas lung-specific overexpression of IL6 accelerates pulmonary vascular disease progression under basal and hypoxic (10% O_2) conditions.[220] Furthermore, smooth muscle–specific ablation of the IL6 receptor protects mice from hypoxia-induced (10% O_2) pulmonary hypertension.[62] In MCT and SU-5416 hypoxia rats and in PAH patients, there is upregulation of the IL6 receptor in PASMCs.[62] Moreover, there is an increased abundance of phosphorylated STAT3, the activated form of the downstream signaling protein of IL6, in PAH PASMCs. In human PASMCs, overexpression of the IL6 receptor has a proliferative effect, mediated via inhibition of apoptosis.[62] In PAH patients, serum IL6 levels are higher than in healthy controls,[22,23,221] and elevated serum IL6 levels are associated with increased risk of mortality.[22,222] In addition, RV dysfunction is more pronounced in PAH patients with higher IL6 levels despite having similar severity of pulmonary vascular disease,[221] suggesting that IL6 may also have direct negative inotropic effects on the right ventricle.

Tocilizumab is an IL6 receptor–blocking antibody that is currently approved for the treatment of rheumatoid arthritis.[223] Tocilizumab has a side-effect profile that includes increased risk of infection, elevated levels of total cholesterol and low-density lipoproteins, liver function abnormalities, and gastrointestinal side effects.[224] The rationale for tocilizumab in PAH is strongly supported by a recent publication. In vitro, the use of an IL6 receptor–neutralizing antibody induces apoptosis in PASMCs incubated with recombinant IL6[62] (Figure 3). Use of ERBF (20S,21-epoxy-resibufogenin-3-formate), a nonpeptide IL6 receptor blocker at a dose of 0.5 mg/kg per day mitigates the severity of pulmonary hypertension in MCT rats in strategies for both prevention (given at time of monocrotaline) and regression (starting 1 week after monocrotaline). In SU-5416 hypoxia rats (10% O_2 for 3 weeks and return to normoxia for 5 weeks), ERBF (0.5 mg/kg per day starting 2 weeks after return to normoxia) significantly reduces PH severity.[62] In both MCT and SU-5416 hypoxia rats, ERBF reduces the abundance of phosphorylated STAT3 in PASMCs, which in turn promotes apoptosis.

Tocilizumab is currently being investigated in the TRANSFORM-UK (Therapeutic Open-Label Study of Tocilizumab in the Treatment of Pulmonary Arterial Hypertension) study (ClinicalTrials.gov identifier NCT02676947). TRANSFORM-UK is a single-arm study that will investigate whether 6 months of treatment with tocilizumab (8 mg/kg monthly) alters pulmonary vascular disease in 21 PAH patients (excluding PAH patients with systemic lupus erythematosus, rheumatoid arthritis, and mixed connective tissue disease) who are classified as NYHA functional class II to IV on stable PAH therapy for 1 month before enrollment.[225] The primary end points will be change in PVR at 6 months and safety, with secondary end points including 6MWD, NT-pro-BNP, symptom burden, and quality of life.[225] This trial has completed enrollment and results should be available soon.

Trimetazidine

As discussed earlier, inhibition of the Randle cycle may be a treatment option for RV dysfunction in PAH. Trimetazidine is an antianginal agent that partially inhibits FAO.[226] In heart failure patients, trimetazidine improves LV ejection fraction and NYHA functional class and reduces hospitalization and mortality.[227–229] Trimetazidine is used in heart failure patients with angina in Europe.[230] Trimetazidine is generally well tolerated, with the most common side effect being gastrointestinal disturbances.[231]

For PAH, trimetazidine has the potential to improve RV function by activating the Randle cycle and increasing glucose oxidation (Figure 5). In PA-banded rats, trimetazidine (0.7 g/L of drinking water) administration starting at the time of PA banding and continuing for 8 weeks normalizes levels of CPT1 (carnitine palmitoyltransferase 1; a key FAO enzyme), Glut1, and hexokinase.[185] Moreover, trimetazidine activates PDH, reduces FAO, and increases glucose oxidation. The metabolic changes in the right ventricle augment cardiac output, dampen RVH, and improve exercise capacity.[185]

Table 3. Summary of Current Indications, Side-Effect Profiles, and Available Clinical Trial Data for Potentially Repurposed Drugs

Drug	Current Indication in Patients	Side Effects	Completed Trial	Dose	Results	Ongoing Trials	Dose	Primary End Point
Aldosterone antagonist	Congestive heart failure (HFrEF), ascites, hypertension	Hyperkalemia, gynecomastia	No	NA	NA	Yes	25–50 mg/d	6MWD, VO_2max, clinical worsening
Allopurinol	Gout, nephrolithiasis	Stevens-Johnson syndrome, nausea	No	NA	NA	NA	NA	NA
Anakinra	Rheumatoid arthritis, refractory pericarditis	Headache, vomiting, immunosuppresion	Yes	100 mg for 14 d	Decreased hs-CRP and reduction in symptom burden	NA	NA	NA
Anastrozole	Adjuvant for breast cancer	Hot flashes, reduced bone mineral density	Yes	1 mg/d	Increased 6MWD	Yes	1 mg/d	6MWD
Apabetalone	Coronary artery disease	Transaminase elevation	No	NA	NA	Yes	100 mg twice daily	PVR
β-Blockers	Congestive heart failure (HFrEF), angina, hypertension, variceal bleed prophylaxis in cirrhosis	Bradycardia, hypotension, fatigue	Yes	Bisoprolol: Up to 10 mg/d Carvedilol: Up to 25 mg twice daily	Bisoprolol: Decreased cardiac index and a trend towards reduced exercise capacity Carvedilol: Reduced RV glucose uptake but no change in cardiac output or exercise capacity	No	NA	NA
Chloroquine	Rheumatological conditions, malaria	Vision disturbance, weakness, nausea	No	NA	NA	No	NA	NA
Colchicine	Gout, familial Mediterranean fever, chronic pericarditis	Diarrhea, peripheral neuropathy, bone marrow suppression	No	NA	NA	No	NA	NA
Dehydroepiandrosterone	Supplement, menopausal symptoms	Acne, excess hair growth	No	NA	NA	Yes	50 mg	RV longitudinal strain on cardiac MRI
DHEA	Inherited mitochondrial disorders	Peripheral neuropathy, fatigue, confusion	Yes	Up to 6.25 mg twice daily	Reduced mPAP and PVR in susceptible patients	No	NA	NA
Metformin	Type 2 diabetes mellitus	Gastrointestinal disturbance, lactic acidosis, fatigue	No	NA	NA	Yes	Unknown	Insulin resistance, oxidant stress markers in urine and plasma, safety

Continued

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Table 3. Continued

Drug	Current Indication in Patients	Side Effects	Completed Trial	Dose	Results	Ongoing Trials	Dose	Primary End Point
Nab-rapamycin	Multiple types of cancer	Thrombocytopenia, fatigue, rash, diarrhea, nausea	No	NA	NA	Yes	Unknown	Safety
Olaparib	Breast and ovarian cancer	Bone marrow suppression, abdominal pain, and nausea/vomiting	No	NA	NA	Yes	400 mg twice daily	PVR
Paclitaxel	Multiple types of cancer	Diarrhea, bone marrow suppression, nausea, peripheral neuropathy	No	No	NA	No	NA	NA
Ranolazine	Refractory angina pectoris due to coronary artery disease	QT prolongation, nausea, dizziness	Yes	500 mg daily 1000 mg twice daily	Improved RV function at exercise with higher dose, no effect with lower dose	Yes	500–1000 mg twice daily	RVEF via cardiac MRI
Rituximab	Non-Hodgkins lymphoma Rheumatological diseases	Immunosuppression, fatigue, injection site reaction	No	NA	NA	Yes	1000 mg 14 d a part	PVR
Rosiglitazone/ pioglitazone	Type 2 diabetes mellitus	Increased risk of heart failure, joint pain, sore throat	No	NA	NA	No	NA	NA
Tacrolimus	Posttransplant immunosuppression	Immunosuppression, renal impairment, hypertension	Yes	Serum levels <2, 2–3, 3–5 ng/mL	Mixed results on 6MWD and RV function which depended on increases in BMPR2 activity	No	NA	NA
Tocilizumab	Rheumatoid arthritis	Immunosuppression hyperlipidemia, liver function abnormalities	No	NA	NA	Yes	8 mg/kg monthly	PVR
Trimetazidine	Angina, congestive heart failure (HFrEF), approved in Europe but not North America	Gastrointestinal disturbance, tremor	No	NA	NA	Yes	35 mg twice daily	RVEF via cardiac MRI
TNF-α-inhibitor	Rheumatological/ autoimmune diseases	Immunosuppression, nonmelanoma skin cancer	No	NA	No	No	NA	NA
Verteporfin	Age-related macular degeneration	Dry-eye, injection site irritation, photosensitivity	No	No	NA	No	NA	NA

BMPR indicates bone morphogenic protein receptor; DHEA, dehydroepiandrosterone; HFrEF, heart failure with reduced ejection fraction; hs-CRP, high-sensitivity C-reactive protein; mPAP, mean pulmonary arterial pressure; MRI, magnetic resonance imaging; NA, not available; PVR, pulmonary vascular resistance; RV, right ventricular; RVEF, right ventricular ejection fraction; 6MWD, 6-min walking distance; TNF-α, tumor necrosis factor α; VO_2max, maximal oxygen consumption.

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The use of trimetazidine in PAH is being investigated in a single-center randomized controlled trial that will examine whether 3 months of trimetazidine treatment (35 mg twice a day) alters RV function in 25 PAH patients. The primary end point will be change in RV function, as quantified by cardiac MRI. Secondary end points will include changes in cardiac fibrosis quantified by T1 cardiac MRI mapping, functional class, and plasma levels of lactate dehydrogenase (ClinicalTrials.gov identifier NCT03273387).

TNF-α Inhibitors

In PAH, the TNF-α pathway is another potential therapeutic target. PAH patients have higher serum levels of TNF-α than healthy controls,[22] and elevated levels of TNF-α are associated with increased bodily pain,[232] suggesting that TNF-α may contribute to systemic symptoms in PAH. Moreover, TNF-α is linked to a reduction in BMPR2 protein abundance in PASMCs[233] and thus has an important molecular target in the pulmonary vasculature.

TNF-α inhibitors are immunomodulators that are used in a wide variety of rheumatological/autoimmune diseases including rheumatoid arthritis,[234,235] psoriasis/psoriatic arthritis,[236] and inflammatory bowel disease.[237] TNF-α inhibitors are generally well tolerated, with side effects including injection site reaction, immunosuppression, and a trend for increased risk of nonmelanoma skin cancer.[238] However, in heart failure patients, use of the TNF-α inhibitor infliximab is associated with clinical worsening in patients with moderate to severe heart failure.[239] In contrast, another TNF-α inhibitor, etanercept, is proven to be safe in heart failure patients.[240,241]

In MCT rats, treatment with etanercept (2.5 mg/kg twice a week) blunts pulmonary vascular remodeling and reduces PA pressures in protocols for prevention (starting the day following monocrotaline injection) and regression (14 days after monocrotaline injection).[233] In pigs with acute endotoxin-induced pulmonary hypertension, etanercept (25 mg) lowers pulmonary pressures and PVR index 4 hours after endotoxin administration.[242] The mechanistic underpinnings of the protective effects of anti–TNF-α therapy on adverse pulmonary vascular remodeling were recently identified. TNF-α decreases BMPR2 protein expression and promotes intracellular accumulation of a cleaved and inactive form of BMPR2 in PASMCs (Figure 4). The reduction in BMPR2 signaling increases PASMC proliferation.[55] Moreover, TNF-α activates NOTCH2 (notch 2) signaling (Figures 2 and 3), which is pro-proliferative, via SRC kinases.[55] Etanercept (2.5 mg/kg twice weekly starting after week 5 of normoxia) in SU-5416 hypoxia rats (3 weeks of 10% O_2 followed by 8 weeks of normoxia) normalizes BMPR2, decreases NOTCH2, and restores activated SMAD in the pulmonary vasculature.[55] These molecular changes result in less pulmonary vascular remodeling, lower RVSP, and moderation of RVH.[55]

No trial is currently under way to examine the effects of TNF-α inhibitors in PAH patients. A strong scientific rigor score of 7 (Table 1), along with the safety profile, suggests that a trial could be considered in the future.

Verteporfin

YAP (Yes-associated protein) is a Hippo signaling molecule that is activated by a stiff ECM and promotes cellular proliferation and survival.[243] YAP induces a metabolic switch to glutaminolysis, an alternative metabolic pathway that utilizes glutamine as an energy substrate and promotes rapid cell growth and hypertrophy.[63] Glutaminolysis is increased in cancer[244] and in PAH[19,245] when cells are exposed to a stiff ECM. Glutaminolysis is also induced de novo in RVH.[245] In MCT rats, primates with simian immunodeficient virus–associated PAH, and PAH patient samples, YAP protein levels are elevated in the pulmonary vasculature.[19]

Verteporfin is a YAP inhibitor that is used clinically in photodynamic therapy to treat neovascularization in age-related macular degeneration.[246] Verteporfin is not used frequently, but the side effects include injection site irritation and photosensitivity.[247] In MCT rats, daily verteporfin (25 mg/kg per day) starting the day after monocrotaline decreases activity of lung lysyl oxidase, a collagen crosslinking enzyme that promotes ECM stiffness, and normalizes pulmonary arteriolar stiffness.[19] In addition, verteporfin reduces mRNA levels of CCN2 and CCN1, 2 YAP-regulated genes. Furthermore, verteporfin treatment blunts the activity of glutaminase, the enzyme that promotes glutaminolysis (Figure 5) in the lungs.[19] Finally, verteporfin treatment mitigates pathological pulmonary vascular remodeling and decreases RVSP and RVH in MCT rats.

The clinical utility of verteporfin in PAH is not being investigated. It may be difficult to use verteporfin in PAH because it is given as an intravenous injection and it has a half-life of only 5 hours.[248] Moreover, it is photoactivated; therefore, it may cause systemic photosensitivity if given chronically. Finally, the preclinical doses greatly exceed those used clinically, and a scientific rigor score of 2 (Table 1) raise questions regarding translatability. Perhaps an inhaled formulation of verteporfin could circumvent the risk of systemic side effects to help realize the beneficial effects of YAP inhibition for PAH.

Conclusions

In this review, we discussed the scientific basis—at both the basic and clinical science levels—supporting the notion that

22 currently available medications may have the potential to improve outcomes in PAH. Furthermore, we highlighted the beneficial effects of anastrozole, dichloroacetate, ranolazine, and tacrolimus that were demonstrated in early phase clinical trials (Table 3). There is always a large barrier in translating data from preclinical models created in rather homogenous rodent populations to human disease in genetically heterogeneous patient populations with multiple comorbid conditions. In addition, studies in rodents often use higher doses of drug than are tolerated by patients (Table 2). Furthermore, many preclinical studies lack the rigor that randomized controlled trials in patients use, notably, absence of blinding, randomization, and careful surveillance for toxicity (Table 1).[81] Moreover, preclinical studies are often brief in duration; this approach fails to mimic the use of the drug in PAH patients, which is often sustained for years. Consequently, preclinical studies may identify molecules that may not be beneficial in patients in clinical trials. These concerns should not lead us to abandon the testing of PAH candidate drugs in preclinical studies; rather, we should increase the rigor of preclinical testing, as recently highlighted.[81] Nonetheless, the fact that these medications are currently used in humans, so the toxicities and drug–drug interactions are already known, could allow for proof-of-principle trials in PAH patients with less risk of adverse effects.

In addition to hypothesis-driven research, use of network analysis combined with patient data shows promise as an unbiased approach for repurposing medications. This method demonstrated a reduction in risk of coronary artery disease in patients treated with hydroxychloroquine.[249] Consequently, this approach may promote discovery of even more drugs that could be repurposed for PAH and help circumvent the barriers that exist when attempting to translate preclinical data to patients by using patient data only.

Although repurposing medications seems to be a safe option, this method still needs to be approached with caution and scientific rigor because repurposing of approved drugs that appear promising in preclinical models can also fail to yield positive results when studied in PAH patients. For example, imatinib reduces the severity of pulmonary hypertension and improves survival in MCT rats[250]; in PAH patients it increases 6MWD and reduces PVR, but it is associated with an increased risk of subdural hematoma that prevents clinical approval.[251] Moreover, although evidence suggests the preclinical efficacy of the selective serotonin reuptake inhibitor fluoxetine,[252–254] the dopamine agonist and serotonin receptor antagonist terguride,[255] and the ASK1 (apoptosis signal-regulating kinase 1) inhibitor[256] selonsertib, none of the clinical trials using these therapies have yielded positive results. Thus, we may need to more carefully choose the medications to repurpose and the patients in whom they are tested.

Perhaps precision medicine will help us identify PAH patients who will benefit from the 22 medications we have discussed. For instance, patients with specific genetic profiles respond to dichloroacetate, whereas those harboring polymorphisms in *UCP2* and *SIRT3* do not,[165] suggesting that genotyping before enrolling in a trial may select a population of responders. Likewise, patients treated with anakinra or tocilizumab could be chosen based on the levels of IL1 and IL6 in peripheral blood rather than enrolling patients without an inflammatory phenotype. Moreover, DHEA may be most beneficial in patients with low serum DHEA levels, and thus these patients could be selected in a clinical trial. Finally, there is precedent for use of genomic profiling to identify PAH patients who may respond to therapy because mRNA levels of *DSG2* (desmoglein 2), a desmosomal cadherin involved in Wnt/β-catenin signaling, and *RHOQ* (ras homolog family member Q), a cytoskeletal protein involved in insulin signaling, in cultured lymphocytes can be used to identify patients who are vasoresponders.[257] The use of genotyping and biomarker profiles are examples of a personalized approach that may enhance the success of translation from preclinical studies to human clinical trials.

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