REGULAR ARTICLE

The Future of PAH Treatment

Jennifer L. Keen, MD
Department of Medicine
Perelman School of Medicine at the University of Pennsylvania
Philadelphia, PA

Nadine Al-Naamani, MD, MS
Department of Medicine
Perelman School of Medicine at the University of Pennsylvania
Philadelphia, PA

Corey E. Ventetuolo, MD, MS
Division of Pulmonary, Critical Care & Sleep Medicine
Brown University
Providence, RI

Pulmonary arterial hypertension (PAH) is a devastating disease mediated by vasoconstriction and vascular remodeling of the pulmonary vasculature. Current therapies target the imbalance of vasoconstrictors and vasorelaxants in 3 pathways: nitric oxide, prostacyclin, and endothelin. While these have extended lifespans for PAH patients, significant morbidity and mortality remains. Notably, the progress in PAH therapy for over a decade has utilized these same 3 pathways. Fortunately, several new treatment options utilizing different mechanisms are emerging and will be reviewed here.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a result of complex pathologic processes culminating in a progressive, incurable disease characterized by elevated pulmonary vascular resistance and right ventricular (RV) dysfunction. Elevated pulmonary afterload derives from both increased vasoconstrictive tone and deranged vascular remodeling that has been likened to a pseudomalignant phenotype. Significant efforts have been made to understand the underlying pathophysiologic processes in the quest for treatment options. Currently approved therapies target 3 pathways—nitric oxide, prostacyclin, and endothelin—as patients with PAH have chronic upregulation of vasoconstrictors such as endothelin and chronic deficiency in vasodilators such as nitric oxide and prostacyclins. However, significant pulmonary vascular disease remains, reflected clinically with an improved but persistently high mortality, particularly in those with high-risk disease.[1] Fortunately, additional pathways involved in the disease have been elucidated and are now candidates for targeted intervention. Many emerging pharmaceuticals target pulmonary vascular fixed remodeling mediated by imbalanced pro-proliferative and antiapoptotic pathways (Figure 1). These potential treatments, together with current therapies, may provide synergistic effects to improve outcomes for our PAH patients. At the same time, ideally we will continue to advance our understanding of precision-based treatments in PAH and move toward the "right drug(s)" for

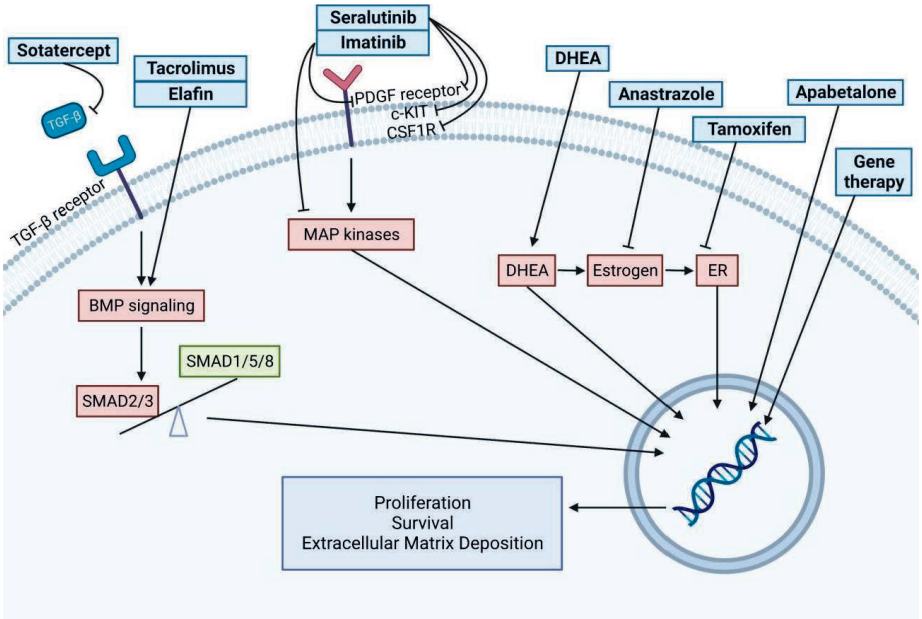

Figure 1: Mechanisms of action of emerging PAH therapies.

Key Words—pulmonary arterial hypertension, novel therapy, drug therapy, clinical trials, growth factors
Correspondence: jennifer.keen@pennmedicine.upenn.edu

Table 1. Summary of Selected New Potential Drugs in the Treatment of PAH

Drug	Mechanism	Trial	Phase	Primary Outcome	Status
Sotatercept	TGF-β ligand trap, BMP signal potentiation	STELLAR	Phase III	Change in 6MWD at 24 weeks	Completed
		SOTERIA	Phase III	Adverse events, detectable anti-drug antibodies, abnormal hematology or chemistry laboratory results, abnormal weight, abnormal blood pressure, ECG, abnormal urinalysis up to 200 weeks	Recruiting
		ZENITH	Phase III	Time to first confirmed morbidity or mortality up to 46 months	Recruiting
		HYPERION	Phase III	Time to clinical worsening	Recruiting
Elafin	Elastase inhibitor, BMP signaling potentiation	*Planned*			
Tacrolimus (FK506)	Calcineurin inhibitor, BMP signaling potentiation	*Planned*			
Imatinib	Tyrosine kinase inhibitor	PIPAH (NCT04416750)	Phase II	Change in PVR at 24 weeks	Recruiting
		IMPAHCT (NCT05036135)	Phase IIb/III	2b: Change in PVR at 24 weeks 3: Change in 6MWD at 24 weeks	Recruiting
Seralutinib (GB002)	Tyrosine kinase inhibitor BMP signaling potentiation	TORREY (NCT04456998)	Phase II	Change in PVR at 24 weeks	Completed
Apabetalone	BET protein inhibitor	APPROACH-2 (NCT04915300)	Phase II	Change in PVR at 24 weeks	Not yet recruiting
Tamoxifen	Estrogen receptor	T3PAH	Phase II	Change in TAPSE at 24 weeks	Recruiting
	inhibitor	(NCT03528902)			
Anastrazole	Aromatase inhibitor	PHANTOM (NCT03229499)	Phase II	Change in 6MWD at 6 months	Completed
DHEA	Steroid hormone precursor	EDIPHY (NCT03648385)	Phase II	Change in RV longitudinal strain on CMRI at 18, 40 weeks	Recruiting
Gene therapy		*Preclinical stage*			
eNOS enhanced progenitor cell transplant	eNOS enhancement	SAPPHIRE (NCT03001414)	Phase II/III	Change in 6MWD at 6 months	Recruiting
Microbiome transfer	Modulating systemic inflammation	NCT04884971	Phase I	Adverse effects and compliance	Recruiting

Abbreviations: TGF indicates transforming growth factor; BMP, bone morphogenetic protein; 6MWD, 6-minute walk distance; ECG, electrocardiogram; PVR, pulmonary vascular resistance; BET, bromodomain and extraterminal motif; TAPSE, tricuspid annular plane systolic excursion; DHEA, dehydroepiandrosterone, RV, right ventricle; CMRI, cardiac magnetic resonance imaging; eNOS, endothelial nitric oxide synthase.

the "right patients" to minimize costly and burdensome regimens and maximize outcomes and quality of life for our patients. It is equally important to validate surrogate endpoints that reflect patient-centered outcomes in PAH trials alongside the discovery of novel mechanistic targets. Readily available PAH risk scores have been suggested as surrogate outcomes in randomized controlled trials of PAH but further validation is required (data unpublished). Identification of alternative validated surrogates is warranted as the efficacy of these emerging potential therapeutics is studied. We will review some of the most promising novel pharmaceuticals currently on the horizon and in clinical trials (Table 1).

BONE MORPHOGENETIC PROTEIN SIGNALING MODULATORS

Disruptions in signaling of the transforming growth factor–β (TGF-β) superfamily contributes significantly to the dysregulated vascular proliferation of PAH. Germline mutations specifically in the TGF receptor of bone morphogenetic protein receptor type 2 (*BMPR2*) and its downstream signalers are the most common genetic cause of heritable PAH,[2] with BMPR2 itself playing a critical gatekeeping role.[3] Bone morphogenetic protein signaling and function is also decreased in nonheritable PAH.[4,5] Downstream of BMPR2, evidence

suggests an imbalance of SMAD signaling with underactive antiproliferative SMAD 1/5/8 signaling and overactive pro-proliferative SMAD 2/3.[6]

Sotatercept

The recombinant fusion protein sotatercept targets this deranged signaling by preferentially inhibiting the pro-proliferative SMAD2/3 pathway. Sotatercept has been previously studied in conditions characterized by TGF-β signaling dysregulation, including multiple myeloma and myelodysplastic syndrome.[7,8] The recent phase II PULSAR trial evaluated sotatercept's efficacy in PAH patients on stable background therapy, at doses of 0.3 mg/kg and 0.7 mg/kg for 24 weeks.[9] The least-squares mean difference of change in pulmonary vascular resistance (PVR) in the sotatercept 0.3-mg/kg group as compared with the placebo group was −145.8 dyn·sec·cm^{-5} (95% confidence interval [CI]: −241.0 to −50.6 dyn·sec·cm^{-5}, $P = .003$), while that for the sotatercept 0.7-mg/kg group compared with placebo group was −239.5 dyn·sec·cm^{-5} (95% CI: −329.3 to 149.7 dyn·sec·cm^{-5}; $P < .001$). Secondary endpoints included improved 6-minute walk distance (6MWD) with least-squares mean difference for the 0.3-mg/kg sotatercept participants compared to placebo participants of 29.4 m (95% CI: 3.8 to 55.0 m) and that of the 0.7-mg/kg group compared to placebo at 21.4 m (95% CI: −2.8 to 45.7 m). Additionally, N-terminal pro-B-type natriuretic peptide (NT-proBNP) decreased with least-squares mean difference between the sotatercept 0.3-mg/kg group and placebo group of −931.5 pg/mL (95% CI: −1353.2 to −509.7 pg/mL) and of the sotatercept 0.7-mg/kg group, −651.0 pg/mL (95% CI: −1043.3 to −258.7 pg/mL).

Multiple phase 3 trials of sotatercept in patients with PAH with varying disease duration and risk profiles are currently ongoing. Early results from STELLAR, a randomized, multi-center study of sotatercept in patients with PAH with World Health Organization (WHO) functional class (FC) II or III on background PAH therapies, were recently reported as meeting the study's primary endpoint of improvement in 6MWD, along with 8 of 9 secondary endpoints including time to clinical worsening, a multicomponent improvement from baseline, maintenance or improvement in WHO FC, change from baseline PVR, change from baseline in NT-proBNP, maintenance or improvement to low risk score, change from baseline in physical impacts, and change from baseline cardiopulmonary symptoms.[10,11] Additional active phase III trials of sotatercept include SOTERIA (NCT04796337),[12] evaluating long-term efficacy and safety; ZENITH (NCT04896008),[13] investigating efficacy in advanced WHO FC III or IV patients on maximally tolerated background therapy; and HYPERION (NCT04896008)[14], studying the drug's efficacy in incident PAH patients.

Elafin

Increased elastase activity has been demonstrated in the pulmonary arteries of experimental PAH models with degradation of elastin, a structural protein that contributes to pulmonary vascular integrity and elastance. Elastin degradation is associated with pulmonary artery smooth muscle cell (PASMC) proliferation.[15] Elafin is a naturally occurring elastase inhibitor with additional antimicrobial and anti-inflammatory properties. Treating pulmonary artery endothelial cells from PAH patients with elafin led to an increase in BMP signaling and a reduction in neointimal formation in cultured pulmonary artery endothelial cells.[16] In the Sugen-hypoxia rat model of PAH, elafin reversed pulmonary vascular occlusive changes and normalized RV pressure.[16,17] These data suggest that elafin may augment BMPR2 signaling in PAH as well as increase expression of apelin, a target of BMPR2 signaling. A small phase I trial (NCT03522935) in healthy patients treated with elafin is complete with plans for a phase 2 proof-of-concept study[18].

Tacrolimus

Tacrolimus, a calcineurin inhibitor used routinely for immunosuppression in transplant patients, also activates BMPR2 signaling.[19] In animal models, low-dose tacrolimus increased BMPR2 signaling in pulmonary artery endothelial cells and reversed pulmonary vascular remodeling in a murine BMPR2 knockout.[19] A small phase 2a trial of tacrolimus at 3 different target levels in PAH patients on background therapy showed no improvement in 6MWD or RV function, but may be efficacious in select patients.[20] Nonetheless, a larger phase II study is being planned to determine the efficacy of tacrolimus more definitively in PAH.

TYROSINE KINASE PATHWAY

Aberrant proliferation of pulmonary vascular smooth muscle cells has been in part attributed to growth factors such as platelet-derived growth factor (PDGF), a potent PASMC mitogen[21] that is increased in PAH patients.[22] PDGF receptors (PDGFRs) belong to a family of tyrosine kinase receptors, and preclinical data have demonstrated that tyrosine kinase inhibitors both attenuate pulmonary vascular remodeling through PDGFR inhibition but also directly relax the pulmonary vasculature.[23] As such, several tyrosine kinase inhibitors are now under clinical investigation for PAH.

Imatinib

Imatinib, a Bcr-Abl inhibitor originally developed to treat chronic myeloid leukemia, also inhibits PDGF. Imatinib potently inhibited PDGF-dependent PASMC proliferation, with near full reversal of pulmonary hypertension in the monocrotaline and hypoxic rat model.[24] Further in vitro data demonstrated that imatinib exerted proapoptotic effects in PDGF-stimulated PASMCs from idiopathic PAH patients.[25] A phase II study of imatinib versus placebo found improvements in PVR and cardiac output specifically in patients with more significant hemodynamic impairment (PVR ≥ 1000 dyn·sec·cm^{-5}), suggesting a role as add-on therapy for a subset of advanced PAH patients.[26] The compelling preclinical data and phase II trial ultimately led to evaluation of imatinib in the IMPRES trial, which enrolled subjects on at least dual background PAH therapies. At 24 weeks, the mean placebo-corrected treatment effect on 6MWD was 32 m (95% CI: 12 to 52 m; $P = .002$) and PVR decreased by 379

dyn·sec·cm^{-5} (95% CI: −502 to −255 dyn·sec·cm^{-5}; P<.001, between-group difference); however, WHO FC, time to clinical worsening, and mortality did not differ between the groups.[27] Serious adverse effects were noted in the imatinib group compared to placebo (44% versus 30%), particularly subdural hematomas, which in addition to significant dropout due to intolerances tempered enthusiasm for imatinib. At present, the ongoing phase II PIPAH trial (NCT04416750)[28] aims to identify the highest tolerated dose of oral imatinib, assess efficacy as measured by PVR reduction, and identify the patients most likely to respond via analyses of plasma proteins and genes, particularly those encoding PDGF. A phase III study of an additional oral formulation of imatinib with enteric coating meant to mitigate gastrointestinal side effects is also planned.[29] Concurrently, IMPAHCT (NCT05036135) is a phase IIb/III trial of dry powder inhaled form of imatinib, which will identify the optimal dose and examine effects of inhaled imatinib on 6MWD and PVR at 24 weeks.[30]

Seralutinib

Seralutinib, which was specifically developed to target several tyrosine kinase inhibitors implicated in PAH pathogenesis including PDGFRα/β, colony stimulating factor 1 receptor, and c-KIT while also increasing BMPR2, has reversed pulmonary vascular remodeling and improved hemodynamics in 2 preclinical pulmonary hypertension models.[31] The phase II TORREY trial of seralutinib delivered by dry powder inhaler was recently completed with early reports of modest improvements in the primary endpoint of PVR at 24 weeks (14.3% placebo-corrected improvement; P = .03) with the secondary endpoint of 6MWD favoring seralutinib as well.[32,33] Findings were more striking in subgroup analyses of more symptomatic WHO FC III patients for the seralutinib arm versus placebo (21% reduction in PVR, P = .04; 37-m improvement in 6MWD, P = .048), and in patients with intermediate- or high-risk REVEAL 2.0 scores (23% reduction in PVR, P = .01; 22-m increase in 6MWD, P = .25) for the seralutinib arm versus placebo.

BROMODOMAIN PROTEINS

Bromodomains (BRDs) are epigenetic drivers of the BRD and extraterminal motif protein family that regulate gene transcription. BRD and extraterminal motif inhibitors may also exert favorable effects on the myocardium and decreased hospitalizations in patients with left heart disease following acute coronary syndrome.[34] BRD4 specifically can inhibit apoptosis, promote hyperproliferation, and stimulate a switch into a proinflammatory phenotype and as such has been implicated in cancer.[35-37] BRD inhibitors have thus been identified as treatment options for cancers.[38] Given the cancer-like proliferation of PASMCs in PAH, it is not surprising that BRD4 has also been identified as a contributor to the proliferation in PAH, with significant upregulation detected in human pulmonary artery tissue.[35]. Accordingly, inhibition of BRD4 reverses pulmonary vascular remodeling and improves hemodynamics in preclinical pulmonary hypertension models.[35] Similar findings were observed in the Phase I APPROACH-p trial of the BRD4 inhibitor apabetalone, with decreased PVR (−140 dyn·s·cm^{-5}; 95% CI: −200 to −79 dyn·sec·cm^{-5}) noted in 7 PAH patients treated for 16 weeks.[39] Improvements in cardiac output (+0.73 L/min; 95% CI: −0.22 to +1.68 L/min) and stroke volume (+8 mL; 95% CI: −4 to +20 mL) were also noted with apabetalone. The larger phase II APPROACH-2 trial (NCT04915300) will confirm or refute these findings.[40]

SEX HORMONES

Estrogen

Despite early identification of female sex as a major risk factor for PAH[41,42] and subsequent intense investigation into sex hormones and their contribution to PAH pathobiology, the exact role of estrogen remains incompletely defined as reviewed more thoroughly in other works.[43–46] Briefly, it is clear that despite female predominance, once PAH is established estrogen provides protective effects on RV function,[47,48] allowing female patients better prognoses.[33,34,41,42,47,49] However, the role of estrogen signaling and metabolism in the pulmonary vasculature itself is complex. Beneficial effects including vasodilation and angiogenesis are noted in some animal studies,[50,51] but other studies report that estrogen promoted destructive vascular remodeling.[52,53] Clinical evidence supports a deleterious relationship with higher circulating estrogen noted in PAH patients, including men.[54] Furthermore, in animal models of PAH, inhibiting estrogen receptors with tamoxifen or inhibiting conversion of androgens to estrogen with anastrazole reversed PAH[55,56] A small phase II clinical trial showed that anastrazole decreased circulating estrogen by 40% and increased 6MWD.[57] This launched the PHANTOM trial (NCT03229499) now underway examining the effects of anastrazole in postmenopausal women and men with PAH.[58] Whether inhibition of estrogen receptors with tamoxifen may benefit PAH patients is also being evaluated with the single-center Phase II T3PAH trial (NCT03528902).[59]

Dehydroepiandrosterone

The precursor to both estrogen and androgens, dehydroepiandrosterone (DHEA) prevented and treated PAH and RV dysfunction in animal models.[60] Clinically, lower DHEA is associated with higher risk of PAH in men[46] and increased risk and severity in women.[61] The consistent data suggesting benefit of DHEA in PAH may be explained by DHEA-mediated enhanced endothelial nitric oxide synthesis or through direct cardioprotective effects. The single-center crossover trial EDIPHY (NCT03648385) is currently testing DHEA efficacy in PAH patients by measuring RV longitudinal strain.[62]

EXPLORATORY THERAPIES

Beyond typical pharmacologic options, other novel approaches are currently under investigation for this complex and morbid disease. As specific gene mutations are implicated in heritable PAH and account for 6% to 10% of all PAH, gene therapy provides an attractive approach to directly correct aberrant genes and restore balance between proliferation and apoptosis. Preclinical pulmonary hypertension models have proven amenable with improvements in

pulmonary vascular remodeling via viral transfection endotracheally and intravenously.[63–65] Work in experimental models is ongoing to determine ideal and effective gene therapy delivery methods.

Stem or progenitor cell therapy may offer similar direct restoration of pulmonary vasculature homeostasis. With the abnormal endothelial dysfunction and hyperproliferation of PASMCs, regenerative cell treatment could interfere and restore vasculature architecture. Endothelial progenitor cells (EPCs) appear protective in PH animal models, including specifically with BMPR2-augmented EPCs, which improved mean pulmonary artery pressures and RV hypertrophy in monocrotaline-induced models.[66] Small pilot randomized controlled trials have demonstrated safety and efficacy of stem cell therapy in humans and a 2019 meta-analysis of 16 small clinical trials with stem cell therapy in PAH patients revealed that despite heterogeneity in findings, weighted-means differences indicated improvements in RV systolic pressure, mean pulmonary artery pressure, and mean RV pressure with P values all $< .001$ in patients treated with stem cells.[67] The PHACeT study in 2015 reported that when treated with 3 doses of enhanced endothelial nitric oxide synthase EPCs, PAH patient demonstrated improved hemodynamics in the short term with good tolerance; however, findings were not sustained at 3 and 6 months,[68] despite prior EPC data showing sustained hemodynamic and exertional effects at 3 months.[69] A recent landmark report described the use of human umbilical cord mesenchymal stems cells to treat a child with heritable PAH and hereditary hemorrhagic telangiectasia which improved clinical parameters at 6 months.[70] Currently the phase II SAPPHIRE study (NCT03001414) is recruiting and aims to assess safety and efficacy of monthly administration of autologous EPCs transfected with human endothelial nitric oxide synthase in severe PAH patients.[71]

Finally, investigation into the microbiome may elucidate novel mechanisms and therapeutic targets in PAH. Compared to controls, PAH patient microbiomes demonstrated decreased alpha diversity with distinct signatures even from unaffected family members, and enrichment of bacteria associated with the proinflammatory metabolite trimethylamine oxide.[72,73] Species associated with trimethylamine oxide were increased as was serum trimethylamine oxide in high-risk PAH patients, whereas species associated with anti-inflammatory metabolites were reduced. Guided by this data, a Phase I trial (NCT04884971) is currently evaluating the safety of microbiome transplant in PAH patients.[74]

CONCLUSION

Despite significant progress in PAH therapeutics over the last 2 decades, innovative treatments are needed to ameliorate morbidity and mortality in this progressive deadly disease. Beyond our current arsenal of treatments, BMP signaling, tyrosine kinase signaling, BRD proteins, sex hormones, and other more novel approaches such as gene therapy targeting pulmonary vascular remodeling are in varied stages of development. With continued scientific rigor used to explore new signaling pathways and mechanisms, we are one step closer to halting, if not reversing, this devastating disease.

References

1. Chang KY, Duval S, Badesch DB, et al. Mortality in pulmonary arterial hypertension in the modern era: early insights from the Pulmonary Hypertension Association Registry. *J Am Heart Assoc*. 2022;11(9):e024969. doi:10.1161/JAHA.121.024969
2. Deng Z, Morse JH, S.L. C, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet*. 2000;67(3):737-744. doi:10.1086/303059
3. Hiepen C, Jatzlau J, Hildebrandt S, et al. BMPR2 acts as a gatekeeper to protect endothelial cells from increased TGFβ responses and altered cell mechanics. *PLoS Biol*. 2019;17(12):e3000557. doi:10.1371/journal.pbio.3000557
4. Dewachter L, Adnot S, Guignabert C, et al. Bone morphogenetic protein signalling in heritable versus idiopathic pulmonary hypertension. *Eur Respir J*. 2009;34(5):1100-1110. doi:10.1183/09031936.00183008
5. Atkinson C, Stewart S, Upton PD, et al. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation*. 2002;105(14):1672-1678. doi:10.1161/01.cir.0000012754.72951.3d
6. Sanada TJ, Sun X, Happe C, et al. Altered TGFβ/SMAD signaling in human and rat models of pulmonary hypertension: an old target needs attention. *Cells*. 2021;10(1) :84. doi:10.3390/cells10010084
7. Abdulkadryov KM, Salogub GN, Khuazheva NK, et al. Sotatercept in patients with osteolytic lesions of multiple myeloma. *Br J Haematol*. 2014;165(8):814-823. doi:10.1111/bjh.12835
8. Komrokji R, Garcia-Manero G, Ades L, et al. Sotatercept with long-term extension for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes: a phase 2, dose-ranging trial. *Lancet Haematol*. 2016;5(2):e63-e72. doi:10.1016/S2352-3026(18)30002-4
9. Humbert M, McLaughlin V, Gibbs JSR, et al. Sotatercept for the treatment of pulmonary arterial hypertension. research-article. *New Eng J Med*.2021;385:1204-1215. doi:10.1056/NEJMoa2024277
10. A Study of Sotatercept for the Treatment of Pulmonary Arterial Hypertension (MK-7962-003/A011-11)(STELLAR): NCT04576988. Updated January 26, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04576988.
11. Merck. Merck announces positive top-line results from pivotal phase 3 STELLAR trial evaluating sotatercept for the treatment of adults with pulmonary arterial hypertension (PAH). https://www.merck.com/news/merck-announces-positive-top-line-results-from-pivotal-phase-3-stellar-trial-evaluating-sotatercept-for-the-treatment-of-adults-with-pulmonary-arterial-hypertension-pah/. Accessed January 6, 2023.
12. A Long-term Follow-up Study of Sotatercept for PAH Treatmnet (MK-7962-004) (SOTERIA): NCT04796337. Updated February 10, 2023. Accessed March 8. 2023. https://clinicaltrials.gov/ct2/show/NCT04796337.
13. A Study of Sotatercept in Participants With PAH WHO FC III or FC IV at High Risk of Mortality (MK-7962-006/ZENITH) (ZENITH): NCT04896008. Updated March 7, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04896008.
14. Study of Sotatercept in Newly Diagnosed Intermediate- and High-Risk PAH Participants: NCT04811092. Updated March 7, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04811092
15. Thompson K, Rabinovitvh M. Exogenous leukocyte and endogenous elastases can mediate mitogenic activity in pulmonary artery smooth muscle cells by release of extracellular-matric bound basic fibroblast growth factor. *J Cell Physiol*. 1996;166(3):495-505. doi:10.1002/

(SICI)1097-4652(199603)166:3<495::AID-JCP4>3.0.CO;2-K.
16. Nickel NP, Spiekerkoetter E, Gu M, et al. Elafin reverses pulmonary hypertension via caveolin-1-dependent bone morphogenic protein signaling. *Am J Respir Crit Care Med*. 2015;191(11):1273-1286. doi:10.1164/rccm.201412-2291OC
17. Zaidi SHE, You X, Ciura S, Husain M, Rabinovitch M. Overexpression of the serine elastase inhibitor elafin protects transgenic mice from hypoxic pulmonary hypertension. *Circulation*. 2002;105(4):516-521. doi:10.1161/hc0402.102866
18. Subcutaneous Elafin in Healthy Subjects: NCT03522935. Updated April 28, 2021. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT03522935
19. Spiekerkoetter E, Tian X, Cai J, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest*. 2013;123(8):3600-3613. doi:10.1172/JCI65592
20. Spiekerkoetter E, Sung YK, Sudheendra D, et al. Randomized placebo-controlled safety and tolerability trial of FK506 (tacrolimus) for pulmonary arterial hypertension. *Eur Respir J*. 2017;50(3):1602449. doi:10.1183/13993003.02449-2016
21. Perros F, Montani D, Dorfmuller P, et al. Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2008;178(1):81-88. doi:10.1164/rccm.200707-1037OC
22. Yu Y, Sweeney M, Zhang S, et al. DGF stimulates pulmonary vascular smooth muscle cell proliferation by upregulating TRPC6 expression. *Am J Physiol Cell Physiol*. 2003;284(2):C316-30. doi:10.1152/ajpcell.00125.2002
23. Rieg AD, Bünting NA, Cranen C, et al. Tyrosine kinase inhibitors relax pulmonary arteries in human and murine precision-cut lung slices. *Respir Res*. 2019;20(1):1-14. doi:10.1186/s12931-019-1074-2
24. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest*. 2005;115(10):2811-2821. doi:10.1172/JCI24838
25. Nakamura K, Akagi S, Ogawa A, et al. Pro-apoptotic effects of imatinib on PDGF-stimulated pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension. *Int J Cardiol*. 2012;159(2):100-106. doi:10.1016/j.ijcard.2011.02.024
26. Ghofrani H, Morrell N, Hoeper M, et al. Imatinib in pulmonary arterial hypertension patients with inadequate response to established therapy. *AmJ Respir Crit Care Med*. 2010;182(9):1171-1177. doi:10.1164/rccm.201001-0123OC
27. Hoeper MM, Barst RJ, Bourge RC, et al. Imatinib mesylate as add-on therapy for pulmonary arterial hypertension. research-article. *Circulation*. 2013;127(10):1128-38. doi:10.1161/CIRCULATIONAHA.112.000765
28. Positioning Imatinib for Pulmonary Arterial Hypertension (PIPAH): NCT04416750. Updated March 1, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04416750
29. Tenax Therapeutics. Clinical Development—The impres trial of imatinib as a treatment of PAH. https://tenaxthera.com/products/imatinib/clinical-development/. Accessed January 15, 2023.
30. A Study of AV-101 (Dry Powder Inhaled Imatinib) in Patients With Pulmonary Arterial Hypertension (PAH) (IMPAHCT). Updated February 17. 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT05036135.
31. Galkin A, Sitapara R, Clemons B, et al. Inhaled seralutinib exhibits potent efficacy in models of pulmonary arterial hypertension. *Eur Respir J*. 2022;60(6):2102356. doi:10.1183/13993003.02356-2021
32. GB002 in Adult Subjects with Pulmonary Arterial Hypertension: NCT04456998. Updated February 9, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04456998.
33. Businesswire. Gossamer Bio Announces seralutinib meets primary endpoint in phase 2 TORREY study in PAH. /. Accessed January 3, 2023.
34. Nicholls SJ, Schwartz GG, Bur KA, et al. Apabetalone and hospitalizations for heart failure in patients following an acute coronary syndrome: a prespecified analysis of the BETonMACE study. *Cardiovasc Diabetol*. 2021;20(1):13. doi:10.1186/s12933-020-01199-x
35. Meloche J, Potus F, Vaillancourt M, et al. Bromodomain-containing protein 4. *Circulation Research*. 2015;117(6):525-535. doi:10.1161/CIRCRESAHA.115.307004
36. Zhang J, Dulak AM, Hattersley MM, et al. BRD4 facilitates replication stress-induced DNA damage response. *Oncogene*. 2019;37(28):3763-3777. doi:10.1038/s41388-018-0194-3
37. Tasdemir N, Banito A, Roe JS, et al. RD4 connects ENhancer remodeling to senescence immune surveillance. *Cancer Discov*. 2016;6(6):612-629. doi:10.1158/2159-8290.CD-16-0217
38. Shorstova T, Foulkes WD, Witcher M. Achieving clinical success with BET inhibitors as anti-cancer agents. *Br J Cancer*. 2021;124(9):1478-1490. doi:10.1038/s41416-021-01321-0
39. Provencher S, Potus F, Blais-Lecours P, et al. BET protein inhibition for pulmonary arterial hypertension: a pilot clinical trial. *J Respir Crit Care Med*. 2022;205(11):1357-1360. doi:10.1164/rccm.202109-2182LE
40. Apabetalone for Pulmonary Arterial Hypertension (APPROACH-2): NCT04915300. Updated April 25, 2022. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04915300
41. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation*. 2010;122(2):64-172. doi:10.1161/CIRCULATIONAHA.109.898122
42. Hoeper MM, Huscher D, Ghofrani HA, et al. Elderly patients diagnosed with idiopathic pulmonary arterial hypertension: results from the COMPERA registry. *Int J Cardiol*. 2013;168(2):871-880. doi:10.1016/j.ijcard.2012.10.026
43. Hester J, Ventetuolo C, Lahm T. Sex, gender, and sex hormones in pulmonary hypertension and right ventricular failure. *Compr Physiol*. 2019;10(1):125-170. doi:10.1002/cphy.c190011
44. Volkmann ER, Siegfried J, Lahm T, et al. Impact of sex and gender on autoimmune lung disease: opportunities for future research: NHLBI working group report. *Am J Respir Crit Crae Med*. 2022;206(7):817-823. doi:10.1164/rccm.202112-2746PP
45. Ventetuolo CE, Ouyang P, Bluemke DA, et al. Sex hormones are associated with right ventricular structure and function: the MESA right ventricle study. *Am J Respir Crit Care Med*. 2011;183(5):659-667. doi:10.1164/rccm.201007-1027OC
46. Baird GL, Walsh T, Aliotta J, et al. Insights from the menstrual cycle in pulmonary arterial hypertension. *Ann Am Thorac Soc*. 2021;18(2):218-228. doi:10.1513/AnnalsATS.202006-671OC
47. Kawut SM, Al-Naamani N, Agerstrand C, et al. Determinants of right ventricular ejection fraction in pulmonary arterial hypertension. *Chest*. 2009;135(3):752-759. doi:10.1378/chest.08-1758
48. Ventetuolo CE, Praestgaard A, Palvesky HI, Klinger JR, Halpern SD, Kawut SM. Sex and haemodynamics in pulmonary arterial hypertension. *Eur Respir J*. 2014;43(2):523-530. doi:10.1183/09031936.00027613
49. Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation*. 2010;122(2):156-163.
50. Umar S, Iorga A, Matori H, et al. Estrogen rescues preexisting severe pulmonary hypertension in rats. *Am J Respir Crit Care Med*. 2011;184(6):712-723. doi:10.1164/rccm.201101-0078OC
51. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest*. 1999;103(3):401-406. doi:10.1172/JCI5347
52. White K, Dempsie Y, Nilsen M, Wright AF, Loughlin L, MacLean MR. The serotonin transporter, gender, and 17β oestradiol

in the development of pulmonary arterial hypertension. *Cardiovasc Res*. 2011;90(2):373-382. doi:10.1093/cvr/cvq408
53. Dempsie Y, Nilsen M, White K, et al. Development of pulmonary arterial hypertension in mice over-expressing S100A4/Mts1 is specific to females. *Respir Res*. 2011;12(1):159. doi:10.1186/1465-9921-12-159
54. Ventetuolo CE, Baird GL, Barr RG, et al. Higher estradiol and lower dehydroepiandrosterone-sulfate levels are associated with pulmonary arterial hypertension in men. *Am J Respir Crit Care Med*. 2016;193(10):168-175. doi:10.1164/rccm.201509-1785OC
55. Chen X, Austin ED, Talati M, et al. Oestrogen inhibition reverses pulmonary arterial hypertension and associated metabolic defects. *Eur Respir J*. 2017;50(2):1602337. doi:10.1183/13993003.02337-2016
56. Mair KM, Wright AF, Duggan N, et al. Sex-dependent influence of endogenous estrogen in pulmonary hypertension. *Am J Respir Crit Care Med*. 2014;190(4):456-467. doi:10.1164/rccm.201403-0483OC
57. Kawut SM, Archer-Chicko CL, DeMichele A, et al. Anastrozole in pulmonary arterial hypertension. A randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med*. 2017;195(3):360-368. doi:10.1164/rccm.201605-1024OC
58. Pulmonary Hypertension and Anastrozole Trial (PHANTOM): NCT03229499. Updated October 21, 2022. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT03229499.
59. Tamoxifen Therapy to Treat Pulmonary Arterial Hypertensin (T3PAH): NCT03528902. Updated August 23, 2022. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT03528902.
60. Lahm, Tuder RM, Petrache I. Progress in solving the sex hormone paradox in pulmonary hypertension. *AmJ Physiol Lung Cell Mol Physiol*. 2014;307(1):L7-26. doi:10.1152/ajplung.00337.2013
61. Baird GL, Archer-Chicko C, Barr RG, et al. Lower DHEA-S levels predict disease and worse outcomes in post-menopausal women with idiopathic, connective tissue disease- and congenital heart disease-associated pulmonary arterial hypertension. *Eur Respir J*. 2018;51(6):1800467. doi:10.1183/13993003.00467-2018
62. Effects of DHEA in Pulmonary Hypertension: NCT03648385. Updated February 21, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT03648385.
63. Ozaki M, Kawashima S, Yamashita T, et al. Reduced hypoxic pulmonary vascular remodeling by nitric oxide from the endothelium. *Hypertension*. 2001;37(2):322-327. doi:10.1161/01.hyp.37.2.322
64. Champion HC, Bivalacqua TJ, Greenberg SS, Giles TD, Hyman AL, Kadowitz PJ. Adenoviral gene transfer of endothelial nitric-oxide synthase (eNOS) partially restores normal pulmonary arterial pressure in eNOS-deficient mice. *Proc Natl Acad Sci USA*. 2002;99(20):13248-13253. doi:10.1073/pnas.182225899
65. Song YK, Liu F, Liu D. Enhanced gene expression in mouse lung by prolonging the retention time of intravenously injected plasmid DNA. *Gene Ther*. 1998;5(11):1531-1537. doi:10.1038/sj.gt.3300770
66. Harper RL, Maiolo S, Ward RJ, et al. BMPR2-expressing bone marrow-derived endothelial-like progenitor cells alleviate pulmonary arterial hypertension in vivo. *Respirology*. 2019;24(11):1095-1103. doi:10.1111/resp.13552
67. Ding XF, Liang HY, Yuan B, et al. Efficacy of stem cell therapy for pulmonary arterial hypertension: a systematic review and meta-analysis of preclinical studies. *Stem Cell Res Ther*. 2019;10(1):55. doi:10.1186/s13287-019-1162-8
68. Granton J, Langleben D, Kutryk MB, et al. Endothelial NO-synthase gene-enhanced progenitor cell therapy for pulmonary arterial hypertension: the PHACeT trial. *Circ Res*. 2015;117(7):645-654. doi:10.1161/CIRCRESAHA.114.305951
69. Wang XX, Zhang FR, Shang YP, et al. Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J Am Coll Cardiol*. 2007;49(14):1566-1571. doi:10.1016/j.jacc.2006.12.037
70. Hansmann G, Chouvarine P, Diekmann F, et al. Human umbilical cord mesenchymal stem cell-derived treatment of severe pulmonary arterial hypertension. *Nat Cardiovasc Res*. 2022;1:568-576. doi:10.1038/s44161-022-00083-z
71. Study of Angiogenic Cell Therapy for Progressive Pulmonary Hypertensin: Intervention With Repeat Dosing of eNOS-enhanced EPCs: NCT03001414. Updated January 11, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT03001414.
72. Kim S, Rigatto K, Gazzana MB, et al. Altered gut microbiome profile in patients with pulmonary arterial hypertension. *Hypertension*. 2020;75(4):1063-1071. doi:10.1161/HYPERTENSIONAHA.119.14294
73. Moutsoglou DM, Tateh J, Prisco SZ, et al. Pulmonary arterial hypertension patients have a proinflammatory gut microbiome and altered circulating microbial metabolites. *Am J Resp Crit Care Med*. 2022. doi:10.1164/rccm.202203-0490OC [online ahead of print].
74. Microbiota Transplant Therapy for Pulmonary Arterial Hypertension: Early Safety and Feasibility Study: NCT04884971. Updated January 10, 2023. Accessed March 8, 2023. https://clinicaltrials.gov/ct2/show/NCT04884971.