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Precision Medicine for Pulmonary Vascular Disease: The Future Is Now (2023 Grover Conference Series)

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Abbreviations: 6MWD, 6-min walk distance; ARDS, acute respiratory distress syndrome; ATAC-seq, transposase-accessible chromatin accessibility with sequencing; BET, bromodomain and extra-terminal domain; BMP, bone morphogenetic protein; BMPR2, bone morphogenetic protein receptor type 2; BPD, bronchopulmonary dysplasia; BRD4, bromodomain-containing protein 4; cAMP, cyclic adenosine monophosphate; CHD, congenital heart disease; COPD, chronic obstructive pulmonary disease; CTEPH, chronic thromboembolic pulmonary hypertension; EFORT, evaluation of prognostic factors and therapeutic targets in PAH; EndoMT, endothelial-to-mesenchymal cell transition; gCap, general capillary; GDF11, growth differentiation factor 11; GDF8, growth differentiation factor 8; HDAC, histone deacetylase; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HIF-1α, hypoxia inducible factor-1α; LASSO, least absolute shrinkage and selection operator; lncRNAs, long noncoding RNAs; MALAT1, metastasis associated lung adenocarcinoma transcript 1; miRNAs, microRNAs; mPAP, mean pulmonary artery pressure; NF-κB, nuclear factor kappa-light chain-enhancer of activated B cells; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAECs, pulmonary artery endothelial cells; PAH, pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PAWP, pulmonary artery wedge pressure; PDGF, platelet derived growth factor; PH, pulmonary hypertension; PPARγ, peroxisome proliferator-activated receptor gamma; PVDOMICS, pulmonary vascular disease phenomics; PVR, pulmonary vascular resistance; REVEAL, registry to evaluate early and long-term PAH disease management; RV, right ventricular; SIN3a, switch-independent 3a; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TET2, ten-eleven translocation methylcytosine-dioxygenase-2; TGF-β, transforming growth factor-beta; TLR4, toll-like receptor 4; WSPH, world symposium on pulmonary hypertension.

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ABSTRACT

Pulmonary vascular disease is not a single condition; rather it can accompany a variety of pathologies that impact the pulmonary vasculature. Applying precision medicine strategies to better phenotype, diagnose, monitor, and treat pulmonary vascular disease is increasingly possible with the growing accessibility of powerful clinical and research tools. Nevertheless, challenges exist in implementing these tools to optimal effect. The 2023 Grover Conference Series reviewed the research landscape to summarize the current state of the art and provide a better understanding of the application of precision medicine to managing pulmonary vascular disease. In particular, the following aspects were discussed: (1) Clinical phenotypes, (2) genetics, (3) epigenetics, (4) biomarker discovery, (5) application of precision biology to clinical trials, (6) the right ventricle (RV), and (7) integrating precision medicine to clinical care. The present review summarizes the content of these discussions and the prospects for the future.

Pulmonary vascular disease is a term that encompasses a spectrum of pathology impacting the pulmonary vasculature. A convergent manifestation of pulmonary vascular pathology is pulmonary hypertension (PH), defined as an elevated resting mean pulmonary artery pressure (mPAP) above 20 mmHg. Epidemiological data are imperfect but PH may affect in excess of 1% of individuals globally [1]. Typically, PH presents with symptoms of exercise intolerance and it is associated with increased mortality [1]. Patients diagnosed with PH are assigned to one of five internationally recognized clinical groups (Table 1). Treatments for Group 1 PH (i.e., pulmonary arterial hypertension [PAH]) have expanded over the last two decades [1] but there is major interest in developing new therapeutic options as well as a particular need for effective treatments for PH assigned to other clinical groups (i.e., non-PAH) and a mission to refine treatment strategies for greater individualized efficacy.

Precision medicine, defined broadly as "providing the right treatment at the right time to the right person," [2] is an aspiration throughout the field of medicine. In reality, it represents a dynamic process that is continually refined [3]. Deep phenotyping is a cornerstone, taking into account features of clinical history including lifestyle factors as well as diagnostic testing, functional assessment, and omics profiling [3]. It benefits from recognizing comorbidity and insights into the underlying molecular drivers of ill health that enable the discovery of biomarkers that inform diagnosis, prognosis, and response to therapy, as well as the development of therapies targeting underlying pathobiology. Precision medicine is bolstered by a growing body of advanced research and the increasing use of novel tools that include omics approaches, computational science, and electronic health records, among others [4].

The 2023 Grover Conference provided an occasion to consider in depth the application of precision medicine to pulmonary vascular disease. New science was presented, key progress was reviewed, and the future was discussed. The present review summarizes the content of these discussions.

1 | Clinical Phenotypes of Pulmonary Vascular Disorders—What We Know and What We Don't Know

Precision medicine relies upon robust clinical phenotyping. Since the first World Symposium on Pulmonary Hypertension (WSPH) in Geneva in 1973 [5] the field has sought to classify PH according to clinical presentation and underlying pathology. The current classification adopted by the 2022 European Society of Cardiology/European Respiratory Society Guidelines recognizes five groups (Table 1), based on hemodynamic measurements obtained by right heart catheterization and the presence of comobidities [1]. Clinical phenotypes as defined by the WSPH classification system are presented, as well as challenges and opportunities to further delineate phenotypes within and across the classification system.

1.1 | Clinical Phenotypes Defined by WSPH Groups

Group 1 PH is a highly heterogenous collection of diseases that range from idiopathic and heritable disease to PH associated with toxins, congenital heart disease (CHD), schistosomiasis, and connective tissue disease (CTD) as well as PAH with features of venous or capillary involvement (e.g., pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis). The current diagnostic paradigm rests upon right heart catheterization (performed among patients with suspected PH) demonstrating precapillary PH (mPAP >20 mmHg with pulmonary artery wedge pressure [PAWP] ≤ 15 mmHg and pulmonary vascular resistance [PVR] >2 WU), with exclusion of

TABLE 1 | Classification of PH.

Group 1: PAH
Idiopathic
Non-responders at vasoreactivity testing
Acute responders at vasoreactivity testing
Heritable
Associated with drugs and toxins
Associated with:
Connective tissue disease
HIV infection
Portal hypertension
CHD
Schistosomiasis
PAH with features of venous/capillary involvement (pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis)
Persistent PH of the newborn
Group 2: PH associated with left heart disease
Heart failure:
With preserved ejection fraction
With reduced or mildly reduced ejection fraction
Valvular heart disease
Congenital/acquired cardiovascular conditions leading to post-capillary PH
Group 3: PH associated with lung diseases and/or hypoxia
Obstructive lung disease or emphysema
Restrictive lung disease
Lung disease with mixed restrictive/obstructive pattern
Hypoventilation syndromes
Hypoxia without lung disease (e.g. high altitude)
Developmental lung disorders
Group 4: PH associated with pulmonary artery obstructions
CTEPH
Other pulmonary artery obstructions
Group 5: PH with unclear and/or multifactorial mechanisms
Hematologic disorders
Systemic disorders
Metabolic disorders
Chronic renal failure with or without hemodialysis
Pulmonary tumor thrombotic microangiopathy
Fibrosing mediastinitis

Note: Adapted from 2022 European Society of Cardiology and European Respiratory Society Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension [1].

other causes of precapillary PH including lung disease and chronic thromboembolic pulmonary hypertension (CTEPH) through history, imaging, and other testing [1]. Among patients with idiopathic, heritable, or drug and toxin-associated PAH, vasodilator i.e. vasoreactivity testing at the time of right heart catheterization is used to identify a subset of patients with favorable response to calcium channel blockers, and is discussed in more detail in the section "Integrating Precision Medicine to Clinical Care." Risk stratification tools further delineate phenotypes according to low-, intermediate- or high-risk disease, incorporating clinical characteristics including functional class, 6-min walk distance (6MWD), and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) [1]. Risk assessment and comorbidities are used to guide therapy, which for most patients relies upon pulmonary vasodilators including phosphodiesterase 5-inhibitors/guanylate cyclase stimulators, endothelin receptor antagonists, and prostacyclin analogs/prostacyclin receptor agonists, and, recently, activin signaling inhibition [1, 6]. Most patients now commence treatment on combination therapy and it is not uncommon to change or add therapy as PH progresses despite best management.

Importantly, the efficacy of therapy in Group 1 PH varies across patients [1]. For example, systemic sclerosis, the most common CTD associated with PAH [7] is associated with a poor response to treatment and worse survival compared to idiopathic PAH or PAH associated with other forms of CTD [8]. Advanced physiological phenotyping using invasive pressure-volume analysis and RV myocardium force-calcium analysis suggests that patients with systemic sclerosis and PAH demonstrate depressed sarcomere function and impaired RV contractile reserve during exercise relative to those with idiopathic PAH [9–11]. Meanwhile, large cohort studies have demonstrated the existence of phenotypes within Group 1 PH which share features of other Groups of PH and impact response to treatment. For example, the "Pulmonary Vascular Disease Phenomics" (PVDOMICS) precision medicine initiative demonstrated that approximately 1/4 of patients with Group 1 PH can also be assigned secondary Groups 2 and 3 contributing conditions [12]. The "Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension" (COMPERA) cohort suggested that, among patients with idiopathic PAH, approximately 1/3 may be characterized by a "left heart phenotype" with shared risk factors for heart failure with preserved ejection fraction (HFpEF) including hypertension, obesity, diabetes, and coronary artery disease, and approximately 1/2 may be characterized by a "cardiopulmonary phenotype" with prior smoking history, hypoxemia, reduced diffusion capacity, and risk factors for left heart disease [1, 13]. When comparing clinical phenotypes, those with idiopathic PAH without left heart or cardiopulmonary features demonstrate the best response to treatment and survival [13] In fact, patients with the cardiopulmonary phenotype demonstrate demographic features, functional impairment, response to PH medications, and survival that is more similar to that of patients with Group 3 PH (e.g., worse treatment response, functional impairment, and survival) than to those with idiopathic PAH without cardiopulmonary features [14].

Group 2 PH is the most prevalent form of PH worldwide and likewise includes a heterogeneous population of patients [1]. Group 2 PH may arise from disease states including HFpEF, heart failure with reduced ejection fraction (HFrEF), valvular heart disease, and congenital conditions contributing to post-capillary PH [1]. It is defined by mPAP > 20 mmHg with PAWP > 15 mmHg [1]. While the presence of PH among patients with left heart disease is common and increases mortality,

there are no recommended PH-specific treatments [1]. In fact, Group 1 PH medications are mostly contraindicated in Group 2 PH due to lack of demonstrated efficacy in randomized controlled trials and in some cases evidence of harm [1]. The less favorable response to pulmonary vasodilators observed in Group 2 PH may relate in part to underlying differences in pulmonary vascular remodeling arising in response to chronically elevated left heart filling pressures. In particular, intimal thickening of pulmonary veins has a strong association with pulmonary artery pressure elevation [15]. In turn, pulmonary vein remodeling can contribute to an increased transcapillary hydrostatic pressure gradient which can predispose patients to pulmonary edema in response to pulmonary vasodilators [15]. Beyond pulmonary vascular remodeling, elevated PAWP increases the pulsatile afterload of the RV which contributes to eventual RV failure [16]. As in Group 1 PH, advanced physiological phenotyping can elucidate distinct physiology and phenotypes. For example, myocardial force analysis of RV cardiomyocytes suggests that patients with PH associated with HFrEF demonstrate reduced myosin response to preload (i.e., less Frank-Starling reserve) relative to controls [17]. Additionally, cardiomyocytes from patients with PH associated with HFrEF with compensated vs decompensated clinical RV function can be differentiated by calcium-activated isometric tension [17]. Meanwhile, among patients with HFpEF, pressure-volume analysis reveals increased RV myocardial stiffness and, during hand-grip exercise, prolonged RV diastolic relaxation which impairs RV filling and limits cardiac output augmentation [18]. The latter suggests that RV diastolic dysfunction contributes to symptoms and pathobiology in HFpEF [18]. Further efforts to phenotype PH due to left heart disease as well as to investigate therapeutics to modulate physiologic abnormalities are needed to improve the poor outcomes associated with Group 2 PH.

In Group 3 PH, patients with PH associated with lung diseases and/or hypoxia are commonly classified according to underlying disease as well as hemodynamic severity (i.e., "non-severe" vs "severe") [1]. A distinct "pulmonary vascular phenotype" among patients with lung disease has been proposed, characterized by mild impairments in spirometry but low diffusion of carbon monoxide, hypoxemia, and exercise limitation [1]. However, even non-severe PH among patients with lung disease is associated with adverse effects on symptoms and survival [1]. In the PVDOMICS cohort, patients with PH associated with lung disease demonstrated poorer prognosis than those with other types of PH [12]. Additionally, a proportion of PVDOMICS participants with predominant Group 3 PH had a mixed phenotype with secondary sources of PH due to comorbidities associated with Groups 1 (20%) and 2 (24%) [12]. Meanwhile, limited treatment options exist. Inhaled treprostinil increased functional capacity among patients with interstitial lung disease [19] but was associated with increased serious adverse events among patients with chronic obstructive pulmonary disease (COPD) [20]. Outside of inhaled treprostinil for patients with PH associated with interstitial lung disease, pulmonary vasodilators are not specifically recommended for Group 3 PH [1]. Nevertheless, many patients with Group 3 PH are prescribed vasodilators [21]. Among patients with PH and COPD enrolled in the COMPERA registry who received treatment with pulmonary vasodilators, 28.5% of patients demonstrated improvement in functional capacity, and patients who demonstrated treatment response demonstrated improved survival compared to those who did not demonstrate treatment response [22]. Coupled with PVDOMICS, such findings suggest the existence of overlapping phenotypes, with some Group 3 patients demonstrating Group 1 treatment response characteristics and vice versa. Further precise phenotyping offers the potential to identify subgroups of patients who may benefit from targeted treatment.

In Group 4 PH, precapillary PH arises from chronic thromboembolic PH or other pulmonary artery obstructions. Approximately 3% of acute pulmonary embolism survivors develop CTEPH [23]. A major challenge relates to a better understanding of the determinants that trigger transition from acute pulmonary embolism to CTEPH. Careful examination of the index computed tomography scan is critical to ascertain signs of already existing CTEPH [24] and to identify completely occlusive acute pulmonary embolism as an imaging phenotype with a threefold increased odds of progression to chronic disease [25]. This progression arises from more than simple persistence of the pulmonary emboli, additionally reflecting distal pulmonary vascular remodeling [23]. Physiologically, this is observed through the greater PVR relative to the degree of macroscopic pulmonary vascular obstruction observed in CTEPH compared to acute pulmonary embolism [23]. The pathophysiology is incompletely understood but depends in part upon shear stress and endothelial dysfunction [23]. Specifically, redistribution of pulmonary blood flow to non-obstructed areas and collateral circulation with systemic pressures to pulmonary vasculature distal to occluded areas are postulated to precipitate microscopic vasculopathy [23]. Emerging evidence suggests a key role of chronic inflammation including macrophage and T-cell modulation of smooth muscle cells in the pathogenesis of microvascular remodeling [26]. Some patients may have symptoms from chronic thromboembolic pulmonary vascular disease without resting PH [27]. Careful exercise testing to ascertain sub-phenotypes of ventilatory inefficiency, exercise-induced PH, or both can suggest potential benefit from treatment with pulmonary endarterectomy or balloon pulmonary angioplasty [27]. However, at present our ability to detect and treat microscopic vasculopathy is limited. Advanced imaging techniques, such as hyperpolarized gas magnetic resonance imaging, may in the future refine our ability to clinically detect microvasculopathy [28]. Additionally, objective tools are needed to define the clinical phenotypes of operable and technically inoperable CTEPH and to identify those at higher risk for residual PH after pulmonary endarterectomy. Such tools may leverage deeper clinical phenotyping, advanced imaging, and/or multi-omic techniques.

Group 5 PH, which includes disease of unclear and/or multifactorial mechanisms, is a heterogeneous group of disorders. Sickle cell disease serves as an example of a Group 5 entity which itself is associated with multiple clinical and hemodynamic phenotypes of PH [29]. PH occurs in 6%–11% of adults with sickle cell disease and increases mortality [30]. Sustained hemolysis with pulmonary vascular iron accumulation and red blood cell and endothelial microparticle-induced endothelial dysfunction contributes to pulmonary vascular disease pathogenesis [31]. Additionally, vaso-occlusive crises with associated tissue ischemia as well as oxidative stress and inflammatory

signaling contribute to pulmonary and systemic vasculopathy [29, 32]. Resultant PH is precapillary in ~40% of patients and postcapillary in ~60% [30] Precapillary PH can represent a Group 1-like phenotype or CTEPH [33] as patients with sickle cell disease suffer a significantly increased risk of venous thromboembolism [30]. Screening for PH through TTE can be challenging in sickle cell disease, as associated high cardiac output may lead to overestimation of tricuspid regurgitation pressure gradient [1]. Even if PH is confirmed through right heart catheterization, treatment strategies are not clearly defined [30]. Case series suggest a possible role for vasodilators in the setting of precapillary hemodynamics, but a randomized controlled trial of phosphodiesterase 5-inhibitor (utilizing TTE but not right heart catheterization for diagnosis of PH) was stopped early due to increased vaso-occlusive crises [30]. Further investigations are needed to differentiate clinical phenotypes of Group 5 PH, including sickle cell disease-associated PH, and to identify targeted therapies [30].

1.2 | Future Directions for Clinical Phenotyping in PH

While the classification system discussed above is the standard of care for categorizing and treating PH, opportunities remain to improve upon clinical phenotyping. The current strategy relies on hemodynamics, yet phenotyping by hemodynamics at a single time point, even when supported by imaging and other clinical data, can struggle to assign almost 40% of patients to a single group [12]. As highlighted above, patients within a Group may share phenotypic characteristics with a separate Group. Additionally, underlying diseases may be associated with multiple Groups of PH. For example, in addition to Group 1 PH, systemic sclerosis is also associated with Groups 2 and 3 PH, where it is associated with particularly poor survival [34, 35]. Group 3 PH includes patients with COPD but COPD may be accompanied by left heart disease and thus some COPD patients may be categorized as Group 2 PH [36]. These observations highlight heterogeneity within the existing PH classification and emphasize the case for more precise phenotyping methods. Modern tools leveraging computational/machine learning approaches to health records, imaging, omics profiling, and other tools offer the potential to identify novel clinical phenotypes [4, 37–39]. For example, proteomic and transcriptomic analyses have identified molecular phenotypes of PAH associated with disease risk and independent from existing Group 1 PH subtypes [38, 39]. A recent network analysis of a large cohort of patients with a mPAP of 19–24 mmHg identified subgroups of patients with shared features and identified pulmonary arterial compliance across subgroups as a key determinant of survival [40]. Ongoing efforts including PVDOMICS seek to integrate deep phenotyping to discover novel endophenotypes and redefine PH classification [41]. The challenge will be to optimize such analysis and integrate it into clinical care [4].

1.3 | Pediatric PH

Pediatric phenotypes of PH represent a special challenge. While pediatric disorders are included in the WSPH classification schemes [1, 42], the heterogeneous nature of pediatric disorders poses challenges to categorization within a traditional adult framework. In 2011 the Pulmonary Vascular Research Institute Pediatrics taskforce developed a comprehensive pediatrics-specific classification system (the Panama Classification) [43]. While the pediatric Panama Classification allows for more precise phenotyping, it still does not fully account for the overlap of multifactorial etiologies that may be somewhat unique to pediatric PH [43]. For example, TBX4-related disease, bronchopulmonary dysplasia (BPD), congenital diaphragmatic hernia, and trisomy 21 offer examples of heterogeneity of PH in infants and children, with contributions from genetics and developmental abnormalities as well as postnatal injury and inflammation. As a result, these conditions may carry physiologic features of WSPH groups 1, 2, and 3 PH across a spectrum of severity (Table 2).

Across heterogenous disorders, abnormal or impaired lung development is a critical contributor to pediatric PH and impacts disease phenotype, severity, and outcomes [54]. Lung development extends from embryogenesis through adolescence [46]. BPD and pediatric acute respiratory distress syndrome (ARDS) represent two pediatric diseases which develop in the setting of pulmonary vascular injury during different stages of lung development. BPD is defined based upon requirements for respiratory support at 36 weeks post-menstrual age in infants born prematurely [55] while pediatric ARDS excludes patients diagnosed with perinatal lung disease [56]. In both diseases, hyperoxia and mechanical ventilation contribute to inflammation, edema, and abnormal growth and repair [55, 57], leading to long-term complications including PH. Survival is better in pediatric compared to adult ARDS, which may relate in part to greater preservation of the pediatric pulmonary endothelial cell barrier function during inflammatory injury [58]. Additionally, some forms of PH in infants and children, such as that associated with BPD, can resolve with age [51] highlighting the unique role that ongoing lung development plays in pediatric PH relative to adult PH. Ultimately, the comparison of clinical phenotypes across a spectrum of pulmonary vascular responses to lung injury, including BPD and pediatric vs adult ARDS, offers the opportunity to investigate overlapping and unique mechanisms that may inform the development of novel therapies.

2 | Genetics of Pulmonary Vascular Disorders: From Family Linkage Analyses to Population-Scale Association Studies

The focus of genetic studies underlying pulmonary vascular pathobiology has been in adult Group 1 PAH. Despite best efforts, less than 1/3 of cases have an identified genetic mutation [59]. The most common gene affected remains that encoding the bone morphogenetic protein receptor type 2 (*BMPR2;* Table 3).

2.1 | BMP Signaling Pathway

The study of families with affected and unaffected individuals pioneered the discovery of disease-causing heterozygous

TABLE 2 | Examples of overlap in pediatric PH.

Condition	Clinical features	Pulmonary vascular pathophysiology	Mechanisms of PH	Outcomes
TBX4-associated disease	Limb (small patella syndrome) and developmental lung disease and airway abnormalities	TBX4 mutation results in disrupted pulmonary vascular development [44]	PAH Developmental lung disease	Associated with PAH, developmental lung disease, and airway abnormalities across a spectrum of severity [44, 45]
Bronchopulmonary dysplasia	Ongoing need for supplemental oxygen and/or respiratory support at 36 weeks post-menstrual age (a metric combining gestational and postnatal age) among infants born preterm [46]	Disrupted pulmonary development (surfactant deficiency, alveolar simplification, and dysmorphic vasculature) [46] and/or lung injury (hyperoxia and/or mechanical ventilation) contributing to inflammation, edema, and abnormal growth and repair [46, 47]	PAH [48] CHD [49] Left ventricular diastolic dysfunction [49, 50] Developmental lung disease [50]	PH is more common in severe disease, and increases morbidity and mortality [50] PH can improve/resolve with time and growth [51]
Congenital diaphragmatic hernia	Developmental diaphragmatic defect, abdominal organ herniation into chest	Lung hypoplasia and impaired lung development, including alveolar simplification, pulmonary arterial muscularization, and left heart hypoplasia and dysfunction [52]	PAH Left heart hypoplasia, left ventricular systolic or diastolic dysfunction Developmental lung disease	PH increases early mortality but can improve with age [52]
Trisomy 21	Down syndrome, including constellation of dysmorphic features, congenital malformations, and intellectual disability	Increased expression of antiangiogenic factors contribute to abnormal lung vascular growth [53] cardiac shunt lesions cause excess pulmonary blood flow and endothelial dysfunction [53]	PAH CHD Developmental lung disease	PH in ~1/4 of children with trisomy 21 [53] Resolution of PH at 3-year follow-up in > 40% of children, with greater likelihood of resolution in those diagnosed at age < 6 months [53]

TABLE 3 | Genes Associated with PAH.

Gene	Pathway/Product	Molecular mechanism	Clinical associations
BMPR2 (Bone morphogenetic protein receptor 2) [60, 61]	TGF-β and BMP	Haploinsufficiency	Mutations observed in 70%–80% of patients with heritable PAH and 10%–25% of patients with idiopathic PAH [62–64]; Penetrance: 14% (males) –42% (females) [62]
ACVRL1 (ALK1; Activin receptor like 1*)* [65, 66]	TGF-β and BMP	Haploinsufficiency	Associated with hereditary hemorrhagic telangiectasia [1]
ENG (Endoglin*)* [65, 66]	TGF-β and BMP	Haploinsufficiency	Associated with hereditary hemorrhagic telangiectasia [1]
SMAD9 (Smad family member 9*)* [65, 66]	TGF-β and BMP	Loss of function	
CAV1 (Caveolin 1*)* [67]	Caveolae	Dominant negative	Associated with lipodystrophy [1]
KCNK3 (Potassium two pore domain channel subfamily K member 3*)* [68]	Potassium channel TASK-1	Loss of function	
EIF2AK4 (Eukaryotic translation initiation factor 2α kinase 4*)* [68]	Stress protein synthesis	Loss of function	Biallelic mutations observed in ~30% of patients with pulmonary veno-occlusive disease, and mono- or biallelic mutations observed in ~2% of patients with PAH [69] Penetrance: nearly complete in pulmonary veno-occlusive disease (autosomal recessive) [59]
ABCC8 (ATP binding cassette subfamily C member 8*)* [70]	Regulatory subunit of potassium channel	Loss of function	
KLK1 (Tissue kallikrein) [64]	Bradykinin		
GGCX (Gamma glutamyl carboxylase) [64]	Posttranslational modification of vitamin K-dependent proteins		
AQP1 (Aquaporin 1) [71]	Aquaporin		
ATP13A3 (ATPase 13A3) [71]	Transmembrane cation transporter		
GDF2 (Growth differentiation factor 2) [71]	TGF-β and BMP	Haploinsufficiency	Associated with hereditary hemorrhagic telangiectasia and CHD [1, 72]
SOX17 (SRY-box transcription factor 17) [71]	Wnt/β-catenin	Haploinsufficiency	Associated with CHD [73]
TBX4 (T-box transcription factor 4) [74, 75]	Fibroblast growth factor	Haploinsufficiency	Associated with TBX4 syndrome and small patella syndrome [73, 76]. Mutations observed in ~1% of adult patients with PAH and ~8% of pediatric patients with PAH [64, 73]
HLA-DPA1/HLA-DPB1 (Major histocompatibility complex, class II, DP alpha 1) [77]	Class II major histocompatibility complex, upstream of *SOX17*		
FBLN2 (Fibulin 2) [78]	Fibulin		

(Continues)

TABLE 3 | (Continued)

Gene	Pathway/Product	Molecular mechanism	Clinical associations
PDGFD (Platelet derived growth factor D) [78]	PDGF		
KDR (Kinase insert domain receptor) [79]	VEGF	Haploinsufficiency	Associated with reduced diffusion of carbon monoxide and later disease onset [71, 79]

Note: *Molecular mechanism is provided where known.

mutations in *BMPR2*, a key receptor of the transforming growth factor-beta (TGF-β) and bone morphogenetic protein (BMP) signaling pathway [60, 61]. It is now estimated that 70%–80% of heritable PAH and about 25% of idiopathic PAH carry pathogenic mutations in *BMPR2* [62, 63]. Longitudinal analysis of families affected by a *BMPR2* mutation demonstrates that females with a *BMPR2* mutation are three times more likely than males to develop the disease with an estimated incomplete penetrance of 42% and 14% in females and males, respectively [62]. Genetic anticipation, or earlier age of diagnosis in subsequent generations, was previously thought to occur in heritable PAH, but more recent analyses suggest a mean age of diagnosis of ~35 years without significant change in subsequent generations [76] *BMPR2* mutation carriers demonstrate a ~2% incidence of PAH/year, and longitudinal studies to-date suggest maintenance of low-risk status in those identified and initiated on treatment following diagnosis through asymptomatic screening [80]. To date, more than 600 pathogenic and likely pathogenic variants in *BMPR2* have been described [81]. The pathogenic role of *BMPR2* in PAH has led to further family-based genetic investigations of other proteins in the TGF-ß and BMP signaling protein-protein interaction network, identifying variants in related genes such as *ACVRL1* (aka *ALK1*), *ENG*, and *SMAD9* [65, 66].

2.2 | Rare Genetic Variants Linked With PAH

Recent exome sequencing studies have identified key rare protein-coding variants with large effect sizes associated with monogenic heritable PAH; namely genes encoding caveolin-1 (*CAV1*) [67] the potassium channel TASK-1 (TWIK-related acid-sensitive potassium channel-1; *KCNK3*) [82] and GCN2 (general control nonderepressible-2, *EIF2AK4*) (Table 3) [68]. The predominant mode of inheritance appears to be autosomal dominant, predisposing to haploinsufficiency [63]. Biallelic variants in *EIF2AK4* underlie pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis [69]. Whole exome sequencing of larger patient cohorts have identified three additional PAH candidate genes; *ABCC8*, encoding ATP-binding cassette subfamily C member 8 [70]. *KLK1*, encoding kallikrein 1, and *GGCX*, encoding gamma-glutamyl carboxylase [64].

The United Kingdom 100,000 Genomes Project (Genomics England Ltd.) undertook whole genome sequencing of over 13,000 individuals, including more than 1000 Group 1 PH cases [83, 84]. The data have enabled the discovery of a number of novel variants as well as common genetic variation associated with PAH. In terms of rare variants, pathogenic or likely pathogenic variation has been observed in: *AQP1*, encoding the membrane channel aquaporin-1; *ATP13A3*, encoding the P-type ATPase 13A3; *GDF2*, a key ligand of the TGF-β signaling pathway bone morphogenetic protein 9 (BMP9); and *SOX17*, encoding the transcription factor SRY-box 17 [71]. *TBX4*, previously associated with PAH and small patella syndrome, was the third most mutated gene in the cohort, triggering additional mechanistic studies [74, 75]. In terms of common variation, two loci associated with PAH; the *HLA-DPA1*/*HLA-DPB1* locus within the class II major histocompatibility complex and a locus upstream of *SOX17* [77]. The latter harbored epigenetic open chromatin and histone modification profiles characteristic for enhancers [77]. Further functional studies in human pulmonary artery endothelial cells (PAECs) confirmed that CRISPR-mediated manipulation targeting the enhancer region specifically repressed *SOX17* expression through differential binding of candidate transcription factors [85]. These findings demonstrate how both rare protein-coding variants as well as common regulatory noncoding variants can affect expression and function of the same gene.

Statistical power is a limiting factor in detecting genotype-phenotype associations. A large international effort involving more than 4000 patients with PAH has identified two additional genes; *FBLN2*, encoding fibulin 2, and *PDGFD*, encoding platelet-derived growth factor D [78]. A Bayesian analysis has been employed to validate a previously suggested association of rare, high-impact loss-of-function variants in the kinase insert domain receptor (*KDR*) gene with significantly reduced transfer coefficient for carbon monoxide and significantly later disease onset [71, 79]. *KDR* encodes the vascular endothelial growth factor receptor 2 (VEGFR2), an essential player for the regulation of angiogenesis, vascular development, permeability, and homeostasis [79].

With a growing number of genetic variants associated with PAH, a working group was formed out of the International Consortium for Genetic Studies in PAH (PAH-ICON, pahicon.com) to evaluate the strength of evidence supporting gene-disease associations using a standardized evidence-based classification system as part of the Clinical Genome (ClinGen) resource. The results of gene-disease association evaluations are communicated with the broader scientific community online (https://clingen.info/affiliation/40071/) and through publications [80, 81].

2.3 | Genetic Variants in Pediatric PAH

While *BMPR2* mutations contribute to pediatric and adult hereditary and idiopathic PAH with similar frequency, rare

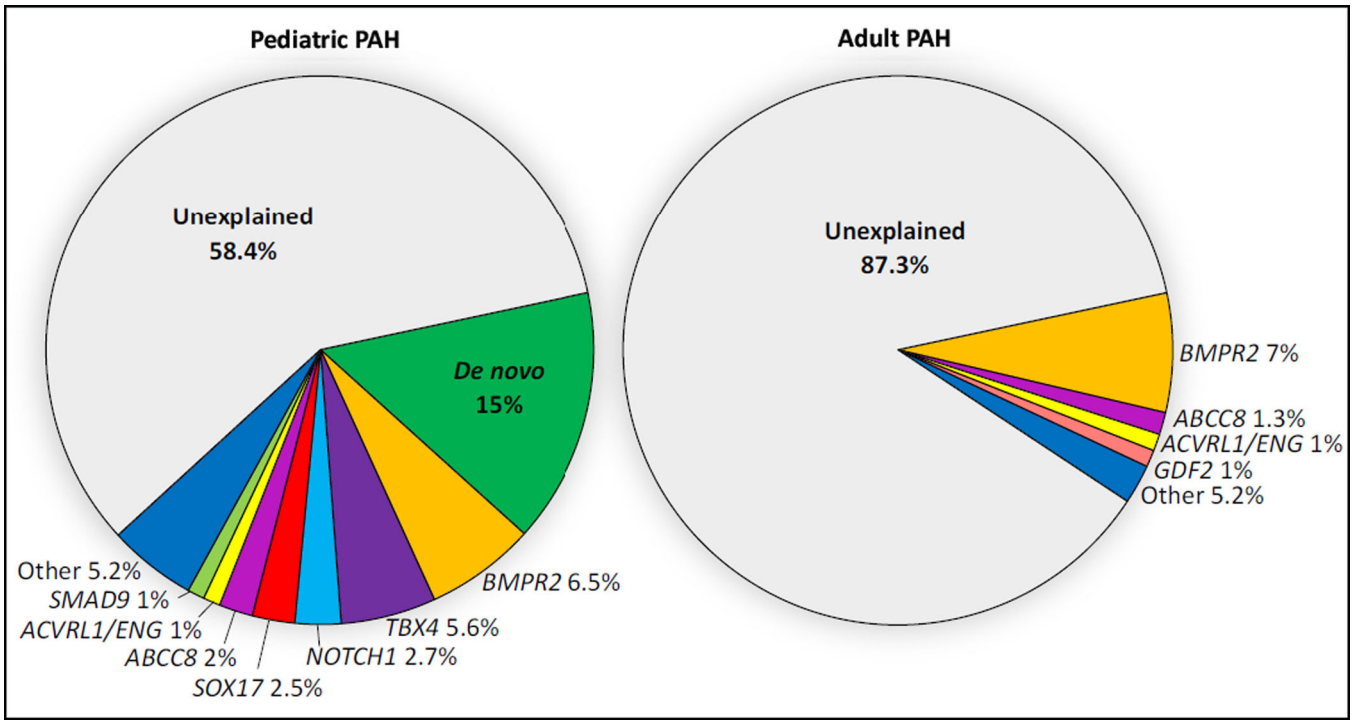

FIGURE 1 | Prevalence of identified genetic factors in pediatric vs. adult PAH. In 443 pediatric and 2628 adult cases of PAH from the Columbia University Irving Medical Center and National Biological Sample and Data Repository for PAH (aka the PAH Biobank) cohorts, de novo and inherited variants were identified in a greater subset of pediatric relative to adult patients. *Figure reproduced from "Genetics and Genomics of Pediatric Pulmonary Arterial Hypertension" by CL Welch and WK Chung, Genes (Basel), 2020 Oct 16;11(10):1213* [88].

genetic variants account for a greater proportion of pediatric idiopathic PAH (Figure 1) [73, 76]. De novo variants in both known risk genes and those not previously implicated in PAH contribute to ~15% of pediatric-onset PAH [73]. The genetic burden is higher in children, owing at least in part to the association with developmental lung disease and CHD. Pathogenic *TBX4* variants are common and account for ~8% of pediatric idiopathic PAH but also are recognized in children with a spectrum of orthopedic abnormalities and lung disease in addition to PH [45, 73, 76, 86]. Genetic variants may also contribute to PH associated with CHD, with recent identification of *SOX17* variants in patients with CHD and pediatric-onset PAH [73]. Patients with *SOX17* variants and CHD may have more severe PAH that presents at a younger age than those without CHD [87]. Overall, pediatric PAH has genetic etiologies which are frequently distinct from adult PAH, and further genetic characterization of pediatric and adult populations is needed to understand the frequency of genetic variants and their full spectrum of contribution to PAH and associated comorbidities.

3 | Understanding Epigenetics and Their Role in Precision Medicine

There is increasing evidence supporting the crucial role epigenetic mechanisms play in mediating gene-environment interactions. Epigenetics encompasses heritable modifications to gene activity that do not alter the primary DNA sequence or genotypes. These modifications include DNA methylation, histone acetylation and methylation, the function of noncoding RNAs including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), and RNA editing. Examples of epigenetic mechanisms are considered with regards to pulmonary vascular disease understanding, biomarker identification, and the potential discovery of novel therapies (Table 4).

3.1 | Epigenetic Mechanisms in Pulmonary Vascular Disease

Multiple noncoding RNAs have been implicated in the pathogenesis of pulmonary vascular disease including through effects on PASMCs [116], PAECs (including endothelial-to mesenchymal cell transition [EndoMT]) [117, 118], and fibroblasts [119]. MiR-130/301 has been proposed to be a master regulator for cell proliferation through signal transducer and activator of transcription 3 (STAT3) and peroxisome proliferator-activated receptor gamma (PPARγ) signaling and can activate PASMC vasoconstriction and cell proliferation [96, 97]. Additionally, miR-130/301 is implicated in a positive feedback loop in heritable PAH by which vascular extracellular matrix stiffening results in further extracellular matrix remodeling [98, 120]. MiR-9 and the lncRNA ribosomal protein S4-like (RPS4L) have been implicated in hypoxia inducible factor-1α (HIF-1α) stimulated PASMC proliferation [89, 106], and the miR-21 family has been implicated in other pathways of hypoxic PASMC proliferation [91, 92]. EndoMT has been shown to be affected by the lncRNA MIR503HG [105] as well as the actions of lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) and miR-145 [99]. The effects of MALAT1/miR-145 are mediated in part through TGF-β receptor type 2 expression [99]. Meanwhile, the miR-17/92 family has been shown to post-

TABLE 4 | Epigenetic modulators with suggested roles in PH.

miRNA/ lncRNA	Function	Cell specificity	Identified diagnostic and therapeutic implications
miRNA			
miR-9 [89]	Regulates proliferation and vasoconstriction under conditions of hypoxia through HIF-1α signaling	PASMC	
miR-17/92 [90]	Regulates proliferation and apoptosis by decreasing BMPR2 protein expression through IL-6/STAT3 signaling	PAEC	
miR-21 [91, 92]	Regulates proliferation and migration under conditions of hypoxia through PPARγ and BMPR2 signaling	PASMC	
miR-26a [93, 94]	Regulates proliferation, migration, and autophagy under conditions of hypoxia through HIF-1α signaling	PASMC	Plasma levels reduced in PAH and correlate with 6MWD [93]; In rats with hypoxia-induced PH, intratracheal administration of adeno-miR-26a-5p improved RV hypertrophy and pulmonary vascular remodeling [94]
miR-124 [95]	Regulates fibroblast glycolysis and proliferation through pyruvate kinase isoform splicing	Pulmonary adventitial fibroblasts	Pharmacologic manipulation of pyruvate kinase isoform activity rescued mitochondrial reprogramming and decreased cell proliferation [95]
miR-130/ 301 [96–98]	Regulates cell proliferation and vasoconstriction through STAT3, apelin, and PPARγ signaling	PASMC, PAEC, and PA adventitial fibroblasts	
miR-145 [99]	Represses EndoMT through TGF-β and SMAD3 signaling	PAEC	
miR-150 [100, 101]	Regulates proliferation and migration under conditions of hypoxia through HIF-1α signaling	PASMC	Plasma levels reduced in PAH and correlate with mortality [100]
miR-204 [102]	Regulates proliferation and apoptosis through STAT3 signaling	PASMC	Delivery of nebulized synthetic miR-204 to lungs of rats with monocrotaline-induced PH resulted in improved hemodynamics [102]
miR-210 [103]	Induces mitochondrial metabolic dysregulation via iron-sulfur cluster assembly repression	PAEC	Extracellular delivery by macrophages results in pulmonary endothelial engraftment, suggesting the potential for non-tissue-specific inhibition to provide tissue-specific benefit [103]
lncRNA			
H19 [104]	Regulates cardiac remodeling through multiple pathways	Cardiomyocytes and cardiac fibroblasts	Plasma levels increased in PAH and correlate with mortality [104]; In monocrotaline and PA banding rat models, suppression reduced RV remodeling and improved RV function [104]
MALAT1 [99]	Promotes EndoMT through TGF-β signaling	PAEC	

(Continues)

TABLE 4 | (Continued)

miRNA/ lncRNA	Function	Cell specificity	Identified diagnostic and therapeutic implications
MIR503HG [105]	Represses EndoMT in part through Polypyrimidine Tract Binding Protein 1-regulated RNA processing	PAEC	Overexpression of MIR503HG in Sugen/Hypoxia mice reduced EndoMT [105]
RPS4L [106]	Regulates proliferation and migration under conditions of hypoxia through HIF-1α signaling	PASMC	
Histone modifications			
HDACs [107]	Represses BMP signaling in hypoxia	PASMC	
Class I HDAC [108]	Represses iron-sulfur biogenesis protein in a HIF-2α dependent mechanism, causing metabolic reprogramming and proliferation	PAEC	
Class I HDAC [109]	Mediates PDGF-induced proliferation and migration	PASMC	Class I HDAC inhibitors counteract PDGF-induced proliferation [109]
Class I HDAC [110]	Mediates pro-inflammatory phenotype	Pulmonary adventitial fibroblasts	Class I HDAC inhibitors decreased cytokine/chemokine expression [110]
BET protein family			
BRD4 [111, 112]	Promotes cell survival and proliferation	Distal pulmonary arteries, PASMCs	Inhibition reduced PASMC proliferation and increased apoptosis and improved pulmonary vascular hemodynamics in experimental models [111,112]
DNA methylation			
SIN3a [113, 114]	Increases BMPR2 promoter region methylation	PASMCs	
TET2 [115]	Demethylates DNA	Peripheral blood monocytes	

transcriptionally downregulate BMPR2 expression in PAECs and thus contribute to PH development [90]. Finally, silenced miR-124 in human and bovine PH pulmonary adventitial fibroblasts contributes to fibroblast metabolic reprogramming via regulation of splicing of pyruvate kinase isoforms, resulting in increased glycolysis and proliferation [95, 121]. Interestingly, in addition to miRNA regulating gene expression within PASMCs, PAECs, and fibroblasts, circulating miRNA may play a role in disease pathogenesis through endocrine effect. For example, the endogenous transport of miR-210 following wildtype bone marrow transplant into miR-210 knockout mice resulted in pulmonary vascular engraftment of miR-210-positive interstitial macrophages and the development of PH [103]. Thus, long-range uptake of miRNAs may play a role in the development of PAH [103].

Changes in histone acetylation through histone acetyltransferases and histone deacetylases (HDACs) have been identified in PAH-associated genes with resultant changes in vascular remodeling [122]. For example, lung tissue from patients with idiopathic PAH demonstrates decreased nuclear HDAC and increased nuclear histone acetyltransferase activity in pulmonary microvascular endothelial cells [123]. HDACs play a role in hypoxia-induced repression of BMP signaling in human PASMCs [107] and HDACs regulate HIF-2α-dependent PAEC metabolic reprogramming which contributes to histological and hemodynamic manifestations of PH [108]. Inhibition or silencing of Class I HDACs in a monocrotaline model of PAH reduced PDGF-induced PASMC proliferation and migration [109] and inhibition of HDACs in a bovine model of PH reduced fibroblast inflammatory signaling and monocyte recruitment [110]. Histone-related epigenetic modifications are also mediated by members of the bromodomain and extraterminal-containing (BET) protein family which "read" acetylated histone tails and facilitate the assembly of transcription complexes [122]. Upregulation of bromodomain-containing protein 4 (BRD4) has been observed in distal pulmonary arteries and PASMCs in PAH patients and contributes to a hyperproliferative, apoptosis-resistant, inflammatory phenotype [111, 112]. Thus, histone modifications contribute to disease pathogenesis through multiple mechanisms targeting multiple pulmonary vascular cells.

DNA methylation is well-documented to contribute to epigenetic regulation of a variety of diseases, and examples have likewise been characterized in pulmonary vascular disease [124]. Methylation-induced attenuation of mitochondrial superoxide dismutase 2 (SOD2) expression has been implicated in a fawn-hooded rat model of PAH to contribute to proliferative, apoptosis-resistant PASMCs [113]. Hypermethylation of

the promoter region of BMPR2 has been observed in patients with heritable PAH [125] and the transcriptional regulator of Switch-Independent 3a (SIN3a) was demonstrated in animal model and human PASMCs to regulate BMPR2 methylation [114]. Mutations in ten-eleven translocation methylcytosine-dioxygenase-2 (TET2), a DNA demethylation enzyme, have been observed in some PAH patients, and decreased TET2 expression was common in a cohort of patients with PAH [115]. In an animal model, TET2 knockout resulted in inflammation and pulmonary vascular remodeling consistent with PAH [115].

3.2 | Potential Role of Epigenetic Modulators as Biomarkers and Therapy

In addition to contributing to disease pathogenesis, epigenetic modulators may serve as a biomarker for risk stratification or molecular classification in PAH. Noncoding RNAs and DNA methylation have potential as biomarkers due to their stability, ease of collection, and detection at low levels via amplification [93, 124, 126]. For example, plasma levels of miR-26a have been demonstrated to be significantly reduced in PAH [93]. Moreover, levels of miR-26a correlated with 6MWD [93]. Plasma levels of lncRNA H19 are increased in patients with PAH relative to healthy controls [104]. H19 appears to have cardiomyocyte-specific effects, and thus provides a unique potential biomarker for RV maladpation [104]. Increased levels identified patients with RV dysfunction, and higher levels predicted worse survival [104]. Finally, plasma levels of miR-150 are reduced in patients with PAH and correlate with mortality [100]. The latter finding was observed through an microarray screen of plasma samples of patients with PAH ($n = 8$) versus healthy controls ($n = 8$) and validated in two larger cohorts of patients with PAH [100]. MiR-150 levels predicted mortality independent of clinical markers of risk [100] suggesting the potential role for miR-150 as a biomarker which can improve upon existing risk stratification. DNA methylation differences in the granulysin gene have been demonstrated between patients with pulmonary veno-occlusive disease versus PAH [127]. Increasing the accessibility of epigenetic marker detection in the future will hopefully lead to larger studies confirming their use as a biomarkers and increasing their role in precision medicine approaches.

Beyond their potential role as disease biomarkers, epigenetic mechanisms have been investigated with respect to therapy. Noncoding RNAs have therapeutic potential due to their small size, conserved sequence, and preclinical evidence in PH and other diseases [128, 129]. Treatment of PASMCs with anti-miR-21 can inhibit hypoxia-induced proliferation [91, 92]. Restoration of miR-200 maintains smooth muscle cell quiescence and represses proliferation, migration, and neointima formation [130]. In rodent models of PH, silencing lncRNA H19 can improve RV hypertrophy and capillary rarefication [104]. Histone modification may also have therapeutic potential. Broad-spectrum HDAC inhibitory molecules such as valproic acid and suberoylanilide hydroxamic acid (vorinostat) prevent and partially reverse PAH in rats through anti-proliferative and anti-inflammatory effects [131]. In animal models, HDAC inhibition can also improve ventricular remodeling [132] although these findings have not been consistent across HDAC inhibitor agents and experimental models [122]. Finally, inhibition of BRD4 has been demonstrated to improve pulmonary vascular remodeling, hemodynamics and RV remodeling in experimental PAH [111, 112]. Despite the promise of utilizing noncoding RNAs, HDAC inhibitors, BET inhibitors, and other therapies leveraging epigenetic effects, the efficacy, delivery method, and minimization of off-target effects require optimization for effective translation to clinical trials [133]. Future work on the role of epigenetics in disease development and testing delivery and efficacy in animal models will assist in overcoming these challenges, hopefully leading to disease-modifying drugs that improve outcomes and decrease mortality. Greater accessibility and further research will be key to realizing the potential of epigenetic approaches to advance precision medicine in pulmonary vascular disease.

4 | Biomarker Discovery for Pulmonary Vascular Disease in the Era of Precision Medicine

Biomarkers that are able to segregate and inform patient management are critical to the pursuit of precision medicine. They are particularly relevant in pulmonary vascular disease, where tissue sampling is rare and often only available at the time of death or transplant. The evolution of “big data” tools including rapid-throughput analytic platforms and machine learning have provided new opportunities to identify disease-specific biomarkers (Figure 2).

4.1 | Biomarker Discovery

Plasma proteomic profiles have been used to identify potential biomarkers for PAH, with a particular focus on predicting outcomes. In a proof-of-concept study, ~1100 plasma proteins were quantified by the SomaScan® platform in patients with idiopathic or heritable PAH [134, 135]. A panel of nine proteins which reflected various features of PAH pathophysiology, including iron deficiency, vascular cell dysfunction, cardiac stress/fibrosis and renal dysfunction, was derived which predicted outcomes independent of the established REVEAL (Registry to Evaluate Early and Long-term PAH Disease Management) risk score [135, 136]. Proteomics platforms are continuing to expand the number of targetable proteins. Two recent studies, again using SomaScan®, harnessed the measurement of over 4000 proteins. In the first, through a discovery and validation design within the UK National Cohort Study of Idiopathic and Heritable PAH, a Cox regression-based model of six proteins was derived which predicted all-cause mortality [37]. This score was formed of proteins that were independently prognostic of 6MWD and NT-proBNP and derived by least absolute shrinkage and selection operator (LASSO) modeling, which penalizes covariates (proteins) contributing the least to the model to reduce the number of variables and avoid overfitting [37]. The model was validated in the French EFORT (Evaluation of Prognostic Factors and Therapeutic Targets in PAH) study of patients with newly diagnosed PAH, and changes in protein score in response to therapy initiation were reflective of altered outcomes [37]. A distinct analysis of healthy controls versus the cohort patients was combined with the prognostic

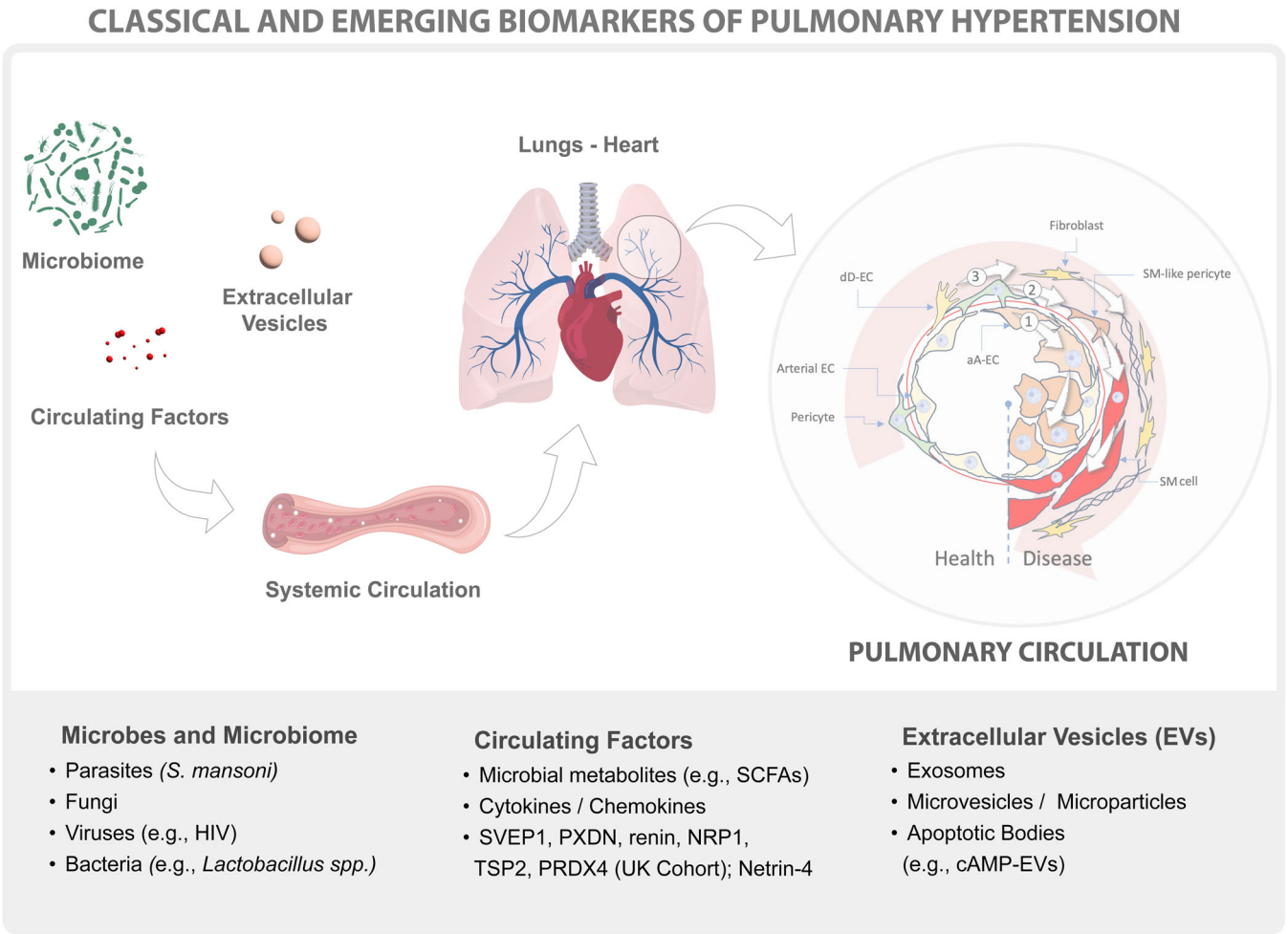

FIGURE 2 | Classical and emerging biomarkers of PH. Several circulating factors such as cytokines, chemokines, and proteins have been classically identified as putative biomarkers of PH. Recently, the range of these biomarkers has broadened to include new categories for early disease diagnosis and disease stage identification. Among them, extracellular vesicles (EVs), the tissue-specific microbiome, and circulating factors associated with microbes and metabolites, have emerged in this expanding group of biomarkers. Abbreviations: cAMP = cyclic adenosine monophosphate; HIV = human immunodeficiency virus; PRDX4 = peroxiredoxin-4; PXDN = peroxidasin homolog; renin, NRP1 = neuropilin-1; *S. mansoni* = *Schistosoma mansoni*; SCFAs = short-chain fatty acids; SVEP1 = Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1; TSP2 = thrombospondin-2.

protein analysis to identify proteins most likely to contribute to PAH pathology [137]. Through a protein genome-wide association study and public data analysis, protein quantitative trait loci were identified for eight of these proteins allowing Mendelian randomization testing, which assessed whether genetic variants associated with protein levels are also linked with the phenotype of interest [138]. This defines the likelihood of proteins being causally associated with PAH. Through this method, netrin-4 was shown to have a causal association between higher plasma levels and PAH whereas thrombospondin-2 was protective, with higher levels representing an ameliorating compensatory response in individuals with PAH [137].

Metabolomics has also been exploited for biomarker discovery. Dysregulated metabolism is observed in PAH both at the level of the pulmonary vasculature and systemically [139]. Metabolite profiles have been demonstrated to correlate with RV function among patients with PAH, and in fact outperform NT-proBNP in predicting RV contractility, diastolic function, and exercise performance [139]. In addition to reflecting RV function, metabolomic profiles have been demonstrated to correlate with REVEAL score and mortality [140]. Within the field of metabolomics, lipidomics provides granularity regarding dysfunctional lipid metabolism, including, for example, the association of distinct lipid profiles with resting and exertional RV function [141].

Omics-based biomarker discovery can be coupled with artificial intelligence computational methods to identify novel patterns within the inherently complex pathobiology of pulmonary vascular disease [4]. Supervised machine learning algorithms use human-provided labels in a dataset with known outcomes to "train" the algorithm [4]. The trained model can then be applied to an unlabeled dataset to predict outcomes [4]. For example, the previously described application of LASSO modeling to identify a protein panel that predicts mortality among patients with PAH represents a form of supervised machine learning [37]. On the other hand, unsupervised machine learning algorithms can be used to infer patterns in an unlabeled dataset [4]. For example, unsupervised machine

learning algorithms have been utilized to identify distinct proteomic immune profiles in PAH [38, 39]. Despite similar demographics, PAH etiologies, comorbidities, and medications across patient clusters, the identified profiles correlated with non-invasive and hemodynamic clinical risk factors as well as survival [38]. A separate investigation applied machine learning to transcriptomics analysis in discovery and validation cohorts of patients with heritable or idiopathic PAH [39]. Transcriptome-associated subgroups of patients were identified with distinct clinical risk factors and survival outcomes [39]. Key differences between subgroups were identified in the expression of *NOG*, a BMP antagonist, and *ALAS2*, a heme biosynthesis enzyme [39]. Additionally, the best- and worst-survival subgroups demonstrated differences in immunoglobulin transcription [39] again suggesting a potential role for immune pathways in differentiating PAH endophenotypes and their biomarkers.

While omics platforms provide powerful tools for biomarker discovery, important limitations exist. Variability in results can result from the use of different tools. For example, only around half of proteins measured on the Olink® protein platform overlap with those detected through the SomaScan® platform. Mass spectrometry-based methods tend to detect only highly expressed molecules. Single-omics approaches provide complex data yet lack the context of interactions between different biological processes. In that respect, multi-omics approaches provide the potential for insights on integrated disease processes. Ultimately, the clinical application of omics-based discoveries will likely require targeted assays—for example, point-of-care lateral-flow tests like those used in pregnancy or COVID-19 rapid antigen testing—to facilitate accessibility and generalizability.

4.2 | Insights Into Vascular Cell Heterogeneity From Single-Cell RNA Sequencing and Spatial Transcriptomics

The growing use of single cell RNA sequencing offers the potential for novel insights into vascular cell heterogeneity as well as vascular cell pathobiology and biomarkers in pulmonary vascular disease. For example, single cell RNA sequencing in a rat Sugen/hypoxia model of PH identified three populations of vascular cells in the spectrum of PH development [142]. First, "activated" arterial endothelial cells emerged during the first week after Sugen administration and were characterized by persistence of differentially expressed genes and a transcriptional profile consistent with growth dysregulation [142]. Growth dysregulation included high levels of expression of *Tm4sf1*, a gene implicated in a number of cancers [142, 143]. Next, classical pericytes were seen to give rise to a smooth muscle-like pericyte cluster that expressed *Actat2* and other contractile proteins [142]. Finally, a "dedifferentiated" endothelial cell cluster was identified, which exhibited loss of expression of endothelial tight junction genes (i.e., *Cldn5*, *Cdh5*), coupled with upregulation of antigen presenting proteins (i.e., *Cd74*) and profound reduction in activity of master transcription factors regulating endothelial cell identity (*ERG1*, *Fli1*) [142]. These findings are summarized in Figure 3. Collectively, this pattern suggests interesting hypotheses regarding EndoMT and adventitial remodeling in pulmonary vascular disease.

Increasingly complex endothelial cell heterogeneity has also been identified at the capillary level. Alveolar capillary endothelium has previously been classified into aerocytes, which contribute to gas exchange and leukocyte trafficking, and general capillary (gCap) cells, which regulate vasomotor tone and serve as progenitor cells in capillary homeostasis and repair [144]. A recent study using high-resolution single cell transcriptomics in endothelial cells isolated from rats and mice identified five distinct gCap populations, each demonstrating unique functional attributes [145]. The associated functions included maintaining the endothelial barrier and structural composition, capillary repair and regeneration, angiogenesis, lipid metabolism, and oxidative phosphorylation [145]. The findings may suggest a role for the capillary vascular beds, rather than pulmonary arteries, in driving PH pathology.

Single-cell RNA sequencing has been used to demonstrate altered angiogenic and mitochondrial processes in PAH PAECs relative to healthy controls [146]. Distinct subsets of PAECs can be distinguished which play causative roles in pathogenic processes known to underly PAH [146]. Among patients with CTEPH, single-cell RNA sequencing demonstrated the presence of macrophages, T-cells and SMCs in thrombus [26]. A dominant macrophage subcluster could be defined by upregulation of inflammatory signaling, and distinct subclusters of T-cells and smooth muscle cells were found with signatures of inflammation and fibrosis [26]. In total, the patterns observed suggest a model of vascular remodeling through macrophage- and T-cell-promoted smooth muscle cell modulation [26].

Spatial transcriptomics offers the potential to complement single-cell analysis with context regarding tissue organization, which is highly relevant to understand the diversity of patterns of pulmonary vascular remodeling in pulmonary vascular disease. In idiopathic PAH, spatial transcriptomics has been used to demonstrate that different types of pathologic lesions (plexiform, obliterative, intimal and medial hypertrophy, and adventitial) express distinct molecular transcript profiles [147]. Plexiform lesions are enriched for genes involved in TGF-β signaling, extracellular matrix formation, and EndoMT [147]. Meanwhile, both plexiform lesions and adventitia demonstrate upregulation of immune signaling, coagulation, and complement pathway genes [147].

Together, the results illustrate the potential of single-cell RNA sequencing and spatial transcriptomics to contribute to our understanding of distinct cell populations and their role in pulmonary vascular disease. Ongoing studies in different experimental models of PH, and in samples from patients with PAH will refine our understanding of pulmonary vascular dysfunction. To the latter point, a novel technique has been described in which PAECs are cultured from pulmonary artery catheter balloons following inflation in the pulmonary arteries during right heart catheterization [148]. Such "cell biopsies" provide the substrate for single-cell omics technology to investigate PAEC heterogeneity in patients [148]. The expectation is that combined analysis of gene expression via single-cell RNA sequencing and spatial transcriptomics, combined with

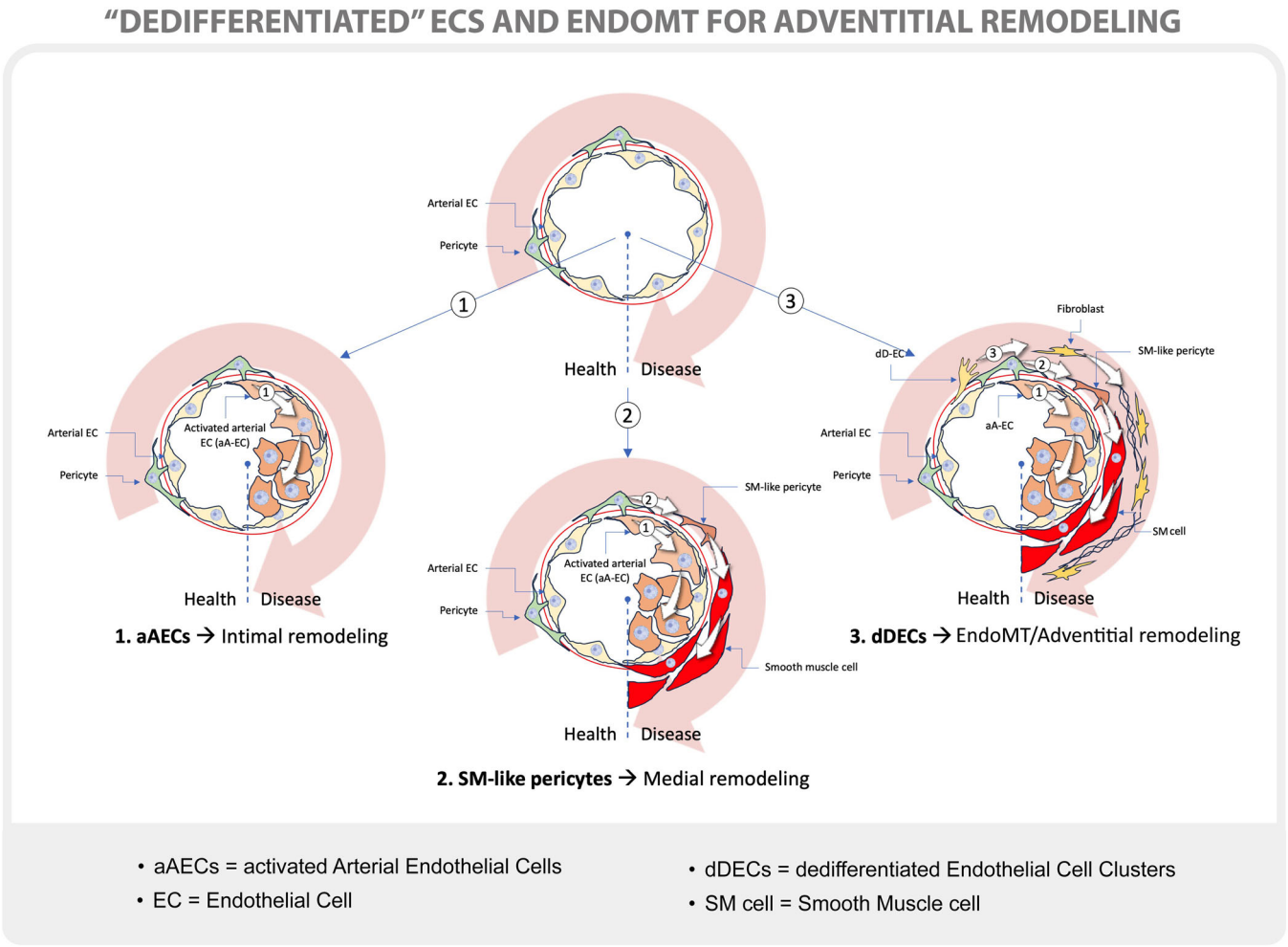

FIGURE 3 | Contributions of "dedifferentiated" endothelial cells to EndoMT for adventitial remodeling. 1. Activated arterial endothelial cells (aAECs) emerge first after Sugen administration, and are characterized by the persistence of differentially expressed genes and a transcriptional profile consistent with growth dysregulation. 2. Classical pericytes give rise to a smooth muscle (SM)-like pericyte cluster expressing contractile proteins. 3. A dedifferentiated endothelial cell cluster (dDEC) emerges, demonstrating loss of expression of endothelial tight junction genes and upregulation of antigen presenting proteins with reduction in activity of master transcription factors regulating endothelial cell activity. This endothelial-to-mesenchymal transition (EndoMT) may contribute to adventitial remodeling.

chromatin accessibility via transposase-accessible chromatin accessibility with sequencing (ATAC-seq) and/or metabolic response via metabolomics will detect dynamic changes in gene regulation and their effect on cellular function. The limitations of these approaches include refining the extent and complexity of data generated for practical application, and machine learning will be an important adjunct tool to identify relevant findings. Nevertheless, in the field of oncology, these tools have been used to discover pathobiology and biomarkers [149] and similar potential exists within pulmonary vascular biology.

4.3 | Extrapulmonary Biomarkers: The Role of the Microbiome in PH

There is increasing recognition of the importance of the microbiome [150–155]. Pathogenic changes in the composition of the microbiome can directly alter tissue function in situ or impact other organs via microbial transposition or microbial-derived metabolites [156]. Microbial metabolites are produced by microorganisms with pro- and anti-inflammatory properties. Among the most common circulating microbial metabolites in the human body, short-chain fatty acids produced by the gut microbiota have emerged as key components of systemic homeostasis. In the lungs, reduced circulating levels of the short-chain fatty acid butyrate have been identified as a contributor to various pulmonary conditions, including asthma, COPD, pulmonary fibrosis, and PH [154, 157, 158].

Microbes and their metabolites can promote cellular effects via the activation of pattern recognition receptors [159]. Toll-like receptor 4 (TLR4) and its co-receptor CD14 have been implicated in animal models of PH and human PH in the gut-lung axis [160, 161]. For example, mice lacking TLR4 expression do not develop PH in response to prolonged hypoxia [162]. Canonically, TLR4/CD14 activates the nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) signaling pathway, leading to the synthesis of pro-inflammatory mediators known to contribute to microbial death but also capable of inducing apoptosis of the host cells, including the cells lining the vasculature. Activation of TLR4/CD14-mediated signaling

in lung vascular endothelial cells results in injury and contributes to the expansion of an abnormal cell phenotype observed in Group 1 PH [163–165].

Microbiome-related molecules may serve as disease biomarkers but also extend to novel therapeutic approaches. Modifying the gut microbiota using antibiotics in the Sugen/Hypoxia rat PH model significantly suppressed vascular remodeling and reduced PH severity [166]. Intermittent fasting in the monocrotaline-induced PH rat model reduced RV hypertrophy and fibrosis and prolonged survival [167]. Such approaches could be combined synergistically with evolving therapies, including mesenchymal stem cell-derived therapy. For example, recent reports showed that mesenchymal stem cell therapy attenuated hypoxia-induced PH in mice by rescuing the gut microbiota composition [168].

Further research is needed to understand and exploit the exact molecular mechanisms of these observations. Future translational studies should carefully account for host-associated factors, such as nutritional habits, co-morbidities, age, sex and associated hormonal factors, and the presence of heritable genetic mutations. Fine manipulation of the host microbiome network offers an interesting avenue for leveraging its novel therapeutic potential and tailoring treatments to individual patients in PH.

4.4 | Extracellular Vesicles: Roles for Biomarker Discovery and Therapy

The term extracellular vesicles encompasses both exosomes and microparticles, which are largely differentiated by their size (exosomes are 50–100 nm; microparticles 100–1000 nm) and protein markers indicative of where within the cell the vesicles are derived [169]. Extracellular vesicles play a role in pulmonary vascular cell–cell communication, displaying surface markers or carrying cargo from their parent cells to target cells [170]. They are released by a wide range of cells within the lungs, including PAECs and fibroblasts [124]. Cargo can include noncoding RNAs and proteins which regulate epigenetic modifications in response to stressors sensed in the lungs such as hypoxia [171]. For example, extracellular vesicles derived from hypoxic PAECs carry increased levels of miR-210-3p and induce PASMC proliferation [172]. Vascular remodeling induced by extracellular vesicle signaling has also been demonstrated in other preclinical models. Extracellular vesicles derived from monocrotaline-injured mice administered to healthy mice induce RV and pulmonary artery hypertrophy [173]. Extracellular vesicles from patients with systemic sclerosis incubated with human PAECs in vitro increase inflammatory cytokines and adhesion molecules [174]. Finally, pulmonary adventitial fibroblasts of calves with severe PH secrete increased levels of extracellular vesicles relative to healthy controls, and the extracellular vesicles mediate metabolic reprogramming and complement-induced inflammatory activation in healthy macrophages [175]. Because of their role in pulmonary vascular disease pathogenesis, extracellular vesicles have been investigated as biomarkers. Levels of circulating endothelial and leukocyte extracellular vesicles are increased in patients with PH [174, 176, 177], and specific extracellular vesicle subtypes correlate with severity of disease as measured by hemodynamics and functional class [176, 177]. Likewise, increased levels of circulating endothelial-derived extracellular vesicles in patients with PH predict a worse prognosis [178]. In oncology, identification of molecules on the surface of extracellular vesicles and noncoding RNA within vesicles has been leveraged for disease diagnosis and monitoring [179]. Further investigation of extracellular vesicles in pulmonary vascular disease, including evaluation of both surface markers and contents, may refine their use as biomarkers.

Extracellular vesicles can also be leveraged as potential cell therapy for PAH. Exosomes or extracellular vesicles from mesenchymal stem cells may contribute to vascular repair without infusion of whole cells [173, 180]. Perhaps somewhat surprisingly, neonatology is one of the fastest moving fields in the adoption of cellular based therapies. Preclinical studies using a rodent model of BPD, namely postnatal hyperoxia exposure, has shown that mesenchymal stem cells prevent alveolar growth arrest, leading to improved airway structure, exercise capacity, and survival [181]. Similarly, mesenchymal stem cell-conditioned media improved pulmonary vascular remodeling and inflammation, thus demonstrating a paracrine effect [182]. Numerous preclinical studies have identified extracellular vesicles as a key component. Derived from the umbilical cord blood and administered intravenously or intratracheally, they are capable of modulating inflammatory responses, vascular remodeling, and alveolarization [183–186]. For example, extracellular vesicles derived from healthy donor mesenchymal stem cells administered to mice with monocrotaline-induced PH have been demonstrated to reverse RV hypertrophy [173]. Mesenchymal stem cell-derived extracellular vesicles administered to mice exposed to hypoxia suppressed hyperproliferative pathways and inhibited pulmonary vascular remodeling [180].

Extracellular vesicles can carry cyclic adenosine monophosphate (cAMP) [170] which can protect the pulmonary vasculature in a number of ways [187]. The administration of cAMP-enriched extracellular vesicles to the hypoxic PH rat model reduced RV hypertrophy and improved echocardiographic RV hemodynamic metrics [188]. Cellular markers of proliferation and pulmonary arterial thickness were also reduced [188]. Combined, these data suggest that cAMP-enriched extracellular vesicles may decrease RV hypertrophy, improve pulmonary arterial function, and repair hypoxic vascular injury [188].

The concept has advanced to clinical evaluation, with the first Phase I trial of allogeneic human umbilical cord blood-derived mesenchymal stem cell transplantation in preterm infants at risk for BPD completed in 2014. In this trial, mesenchymal stem cells were administered intratracheally to nine preterm infants at high risk for BPD or death. The intervention was generally well tolerated [189]. A subsequent Phase II double-blind, randomized placebo-controlled study also demonstrated safety, with a potential treatment benefit in the most extremely preterm patients (23–24 weeks gestation) [190, 191]. Currently, more than two dozen clinical trials are either underway or about to begin worldwide, exploring cell-based therapies for the prevention or treatment of BPD. Optimal administration

strategies—including dosage, route, and frequency—remain uncertain. Additionally, it is unclear whether whole-cell therapies or cell-free alternatives (such as exosomes or extracellular vesicles) are more effective, which has significant implications for pharmaceutical production and scalability. Perhaps most critically, the outcome measures for assessing the success of clinical trials have yet to be clearly defined. This issue was underscored in a recent negative trial of mesenchymal stem cells in non-human primates, where the primary endpoint—improved lung alveolarization—was not achieved. However, infant baboons that received mesenchymal stem cells showed improved cardiovascular stability and required less hemodynamic support [192]. The pleiotropic effects of mesenchymal stem cells and their extracellular vesicles warrant further investigation, making this an exciting time for the development of cell-based therapies for lung diseases, including pulmonary vascular disease.

5 | From Precision Biology to Clinical Trials: Examples for How Basic Research Accelerates Clinical Trials in Pulmonary Vascular Diseases

The advent of inexpensive, readily accessible, and scalable omics technologies enables the interrogation of pulmonary vascular disease on an unprecedented scale. Harnessing this wealth of new information for novel therapeutics is a formidable challenge. Examples from recent and ongoing efforts in drug development were used to consider the present status and future opportunities of "big data" in drug development (Table 5).

TABLE 5 | Examples of novel drug development in PAH.

Drug	Mechanism of action	Clinical trial findings
Sotatercept (ACTRIIA-Fc)	Activin signaling inhibitor [193]	Phase 2: Among patients with PAH in functional class 2–3, sotatercept at 0.3 mg/kg ($n = 32$) and 0.7 mg/kg ($n = 42$) vs. placebo ($n = 32$) improved PVR (primary outcome) and 6MWD and NT-proBNP levels (secondary outcomes) [194] Phase 3: Among patients with PAH in functional class 2–3, sotatercept titrated to 0.7 mg/kg ($n = 163$) vs. placebo ($n = 160$) improved 6MWD (primary outcome) and multiple secondary outcomes [6]
Apabetalone (RVX208)	BRD4 inhibition [111]	Phase 1: Among patients with PAH in functional class 2–3 ($n = 7$), RVX208 was tolerated without serious adverse events. Improvements were observed in secondary outcomes of PVR, cardiac ouptut, and RV ejection fraction [195] Phase 2 study planned (NCT04915300)
Imatinib	Tyrosine kinase inhibitor [196]	Phase 2: Among patients with PAH in functional class 2–4 randomized to imatinib ($n = 28$) vs. placebo ($n = 31$), there was no change in 6MWD but a signficant improvement in PVR and cardiac output among patients treated with imatinib. Serious adverse events were recorded in 39% of patients treated with imatinib vs 23% of patients treated with placebo [197] Phase 3: Among patients with PAH in functional class 2–4 on ≥ 2 pulmonary vasodilators randomized to imatinib ($n = 103$) vs. placebo ($n = 98$), there was a significant improvement in 6MWD and PVR. Serious adverse events, including subdural hematoma, and discontinuations were more frequent among patients treated with imatinib (44% vs. 30%, and 33% vs. 18%, respectively) [198] Phase I/II: A Bayesian design using the Continuous Reassessment Method to identify the best tolerated dose of imatinib (Part 1), followed by evaluation of efficacy of the best tolerated dose (Part 2), is planned [199]
Seralutinib	Tyrosine kinase inhibitor [200]	Phase 2: Among patients with PAH in functional class 2–3 randomized to seralutinib ($n = 44$) vs. placebo ($n = 42$), a reduction in PVR was observed with seralutinib. The most common treatment-emergent adverse events in both groups was cough [201] Phase 3: Study underway (NCT05934526)
Rituximab	Anti-CD20/B-cell depletion [202]	Phase 2: Among patients with SSc-PAH diagnosed for no more than 5 years randomized to rituximab ($n = 29$) vs. placebo ($n = 28$), a nonsignificant improvement in 6MWD was seen with rituximab. There was no significant increase in serious adverse events. Low Rheumatoid Factor levels, IL-12, and IL-17 predicted favorable response to rituximab by 6MWD [203]

5.1 | Sotatercept

Sotatercept is a novel fusion protein (ACTRIIA-Fc) that has been developed as a selective GDF8/11 and Activin-A circulating ligand trap with extensive evaluation in clinical studies in patients with PH. The conceptual paradigm for sotatercept arises from the observation of increased expression of Growth Differentiation Factors (GDF8 and GDF11) and Activin A in PAH [204]. This imbalance is potentially targetable, and the novel fusion protein ACTRIIA-Fc was developed as a selective GDF8/11 and Activin-A circulating ligand trap [193]. In a pivotal preclinical study, ACTRIIA-Fc improved multiple measures of PH disease severity (mPAP, echocardiographic RV systolic pressure, RV hypertrophy, and the degree of pulmonary vascular remodeling) in monocrotaline and Sugen/Hypoxia rodent models of experimental PH [193]. In the PULSAR and STELLAR trials, patients who were randomized to sotatercept had a significant improvement of PVR and 6MWD at 24 weeks compared to patients randomized to placebo [6, 194]. Patients who were randomized to sotatercept in STELLAR also demonstrated improvement in multiple secondary endpoints, including PVR, NT-proBNP, World Health Organization Functional Class, composite PAH risk score, and time to death or clinical worsening [6].

While some long-term data are available [205] further clinical experience with the drug is needed. There remains a concern that telangiectasias and bleeding events may pose a risk and that some individuals may progress to a hereditary-hemorrhagic-telangiectasia-like phenotype. Preclinical data suggests that ACTRIIA-Fc and other ligand traps that block BMP10 mimic hereditary hemorrhagic telangiectasia in experimental PH and susceptible rodent strains [206] and telangiectasias were observed in 10% of patients receiving sotatercept in STELLAR while bleeding events (predominantly non-serious epistaxis) were observed in 22%. The long-term implications as well as considerations of alternate dosing regimens (e.g., roles for “induction” and “maintenance” strategies vs. continuous regimens) are of interest. There is also uncertainty regarding the correct timing for this medication in the modern PAH armamentarium.

Nevertheless, sotatercept represents a recent example of successful drug development. Importantly, the development of sotatercept identified a targetable mechanism rooted in human genetics [193] integrated a range of data sources including established pharmacodynamics from prior human subject experience [207] confirmed efficacy across PH models [193] and harnessed collaboration between the sponsor, investigators, and the PH community. That said, integration of large-scale omics data and systems biology was not part of the development of sotatercept, nor has the focus on mechanism been utilized to-date to determine a biologic subset of individuals more likely to benefit.

5.2 | BRD4

As discussed in the “Understanding Epigenetics and their Role in Precision Medicine” section, examination of human lung tissue from PAH and control subjects has identified increased levels of BRD4, a BET family protein, in the distal pulmonary arteries [112]. Inhibition of BRD4 in PAH PASMCs decreased proliferation and cellular survival, and BRD4 inhibition in the Sugen/Hypoxia rodent model improved PH disease severity as measured by RV hypertrophy, RV systolic pressure, and vascular remodeling [112]. While non-specific inhibition of BET through the inhibitor I-BET-151 in healthy rodents was disappointing and resulted in mitochondrial swelling, disorganized cardiomyocyte structure, and impaired cardiac function [208], studies of selective inhibition of BRD4 reinforced the early impression of benefit [111]. In multiple rodent PH models (Sugen/Hypoxia, monocrotaline and shunt, and pulmonary artery banding), inhibition of BRD4 by RVX208 (apabetalone) decreased PASMC and endothelial cell proliferation and improved PH hemodynamics and vascular remodeling [111]. This success paved the way for a small clinical trial of RVX208 as add-on therapy in PAH [195]. In this open-label, 16-week, single-arm study of seven patients with PAH, RVX208 was safe and well tolerated, with improvement in PVR, cardiac output, stroke volume, and cardiac magnetic resonance imaging measures of RV ejection fraction and both end-systolic and end-diastolic volumes [195]. Given the favorable safety and efficacy signals, a larger randomized clinical trial of RVX208 in PAH is planned (APPROACH-2; NCT04915300).

The rapid development of the BRD4 paradigm reinforces several key principles in current drug development, including the use of multi-center collaborations to reinforce signals from both clinical and pre-clinical data and the use of repurposed but mechanism-targeted drugs (in this case from oncology and atherosclerotic vascular disease). The evolution of the BRD4 paradigm has incorporated a systems biology approach to identifying on-target and off-target impacts over the course of defining and refining compounds of interest.

5.3 | Tyrosine Kinase Inhibitors

First targeted over two decades ago [196] inhibition of the platelet derived growth factor (PDGF) pathway continues to attract interest. Imatinib, licensed for chronic myeloid leukemia, ameliorates PH in both monocrotaline-induced rat and chronic hypoxemia mouse models in a dose-dependent fashion [196]. Efficacy in patients with PAH has been investigated in two randomized placebo-controlled studies [197, 198], but improvements in hemodynamics and 6MWD seen with 400 mg daily were accompanied by safety concerns. An ongoing study is revisiting oral imatinib using a Bayesian study design to explore the safety and efficacy of doses between 100 and 400 mg daily, as well as to explore response based on a common polymorphism (rs2304058) in the gene encoding PDGFRB [199]. Studies of inhaled imatinib are also in progress, with the aim of delivering a therapeutic dose of the drug to the lung that spares systemic side effects.

The recent results from another PDGFR antagonist, seralutinib, support this approach [200, 209]. Seralutinib ameliorates PH disease severity in both Sugen/Hypoxia and monocrotaline-pneumonectomy rodent models in a dose-dependent fashion [200] The Phase II TORREY randomized placebo-controlled double-blind study of seralutinib in PAH has reported a

decrease in PVR [201] and data from a Phase 3 study are awaited. As with sotatercept, there remains work to be done to see if there is a subgroup of patients who might be better targeted with this class of drugs.

5.4 | Rituximab

A post-hoc analysis of a trial of rituximab in scleroderma-associated PAH is an example of the interest in 'responder analysis' to guide patient selection for trials. The rationale for the original study was the observation of lymphoid aggregates with B-cell infiltration in the vascular lesions of PAH, coupled with the fact that B-cell depletion via anti-CD20 therapy improved PH severity and vascular remodeling in both monocrotaline and Sugen/Hypoxia rodent models [202, 210]. A Phase II randomized placebo-controlled double-blind trial of rituximab suffered a number of logistical issues and failed to meet its primary (change in 6MWD) or exploratory secondary endpoints [203]. A post hoc machine learning clustering analysis identified a subset of patients with reduced circulating B-cell specific cytokines as having the best response to treatment [203]. It was subsequently reported that soluble markers of B-cell activation may not only correlate with development of PAH in scleroderma patients, but also with metrics of disease severity (NT-proBNP, and RV systolic pressure), implying that the success of anti-CD20 therapy in scleroderma PAH may just be a matter of identifying the correct patient population and targeting this group more effectively [211, 212]. There are challenges in identifying responders based on a single drug exposure; ideally, patients should be rechallenged to confirm their response. However, using a mechanism-specific target to enrich and define a treatment population represents one of the best examples of precision medicine applied to drug development in PAH.

5.5 | Summary

These examples from recent and ongoing drug development highlight a number of key points at the intersection of precision medicine, precision biology, and drug development in PAH. First, it is still early days. None of the current examples have relied on large-scale integration across multiple omics domains for target identification. At best, traditional family genetic studies highlighted the BMP/TGF-β pathway as a driver of PAH, leading to the repurposing of sotatercept for PAH patients. The full impact of omics on precision phenotyping is yet to be realized, but as seen with drugs like imatinib and rituximab, it is expected to play a critical role in identifying and characterizing responders, selecting likely candidates for treatment (maximizing benefit while minimizing harm), and guiding follow-up studies. Drug repurposing also deserves attention, with the evolution of BRD4-targeting therapies being particularly noteworthy. This would not likely be possible without the emerging systems biology framework to contextualize early results, exponentially expanding drug libraries to allow nuanced compound-specific pivots even within the same pathway, and high-dimensional analyses to better understand populations that may be more or less likely to benefit. Although we have only begun to explore its potential, it is likely that the next set of PAH therapeutics and therapy-specific endotypes will be informed by precision biology and precision medicine insights gleaned from multi-omic dataset integration, big-data and network analysis approaches, and continued understanding of pulmonary vascular diseases as multi-organ systemic disease states.

6 | The RV: A Phenotype of Its Own

The last 5 years have seen major advances in our understanding of mechanisms of RV adaptation and maladaptation in PH. While initial studies in RV failure research primarily focused on animal models, the field is now pursuing in-depth analysis in human tissues. These mechanistic studies are paralleled by advanced and multimodal phenotyping methods in patients with RV failure.

6.1 | Cellular Landscape and Omics Approaches to the RV

According to a recent single cell/single nucleus RNA sequencing study, the most common cell type in the human LV is the fibroblast (~26%), followed by endothelial cells and cardiomyocytes (~17%), as well as macrophages (~12%) [213]. Not surprisingly, several sub-types of all these major cell types exist with up to ten cell types having been described for cardiomyocytes and endothelial cells [214, 215]. Factors such as age, sex, and disease development modify these numbers [214, 215]. How these numbers hold up for the human RV remains to be determined.

Recently published studies employed integrated RNA sequencing and proteomic analyses in RV tissues and plasma from animal models as well as patients with adaptive and maladaptive RV remodeling [216–218]. In the first study, latent TGF-β binding protein 2 was identified as a novel plasma biomarker for RV dysfunction and predictor of survival in patients with PAH (with higher plasma levels indicating maladaptive RV remodeling and a higher likelihood of death) [216]. Other mediators upregulated at both the RNA as well as protein level included *Col18A1*, *TNC*, *Col6A3* and *CA1*, indicating regulators of the extracellular matrix as potential key players in the progression to RV failure [216]. Another study used a similar approach in animal models and human RV tissue, reporting that adaptive and maladaptive RV remodeling stages can be further categorized into early and late stages based on their genomic and proteomic signature [217]. In particular, five extracellular matrix proteins (*NID1, CRTAC1, C1QTNF1, MEGF9, SPARCL1*) distinguished early- to late-decompensated RV remodeling states [217]. Finally, in a bovine model of early RV adaptation to severe hypoxia-induced PH, RV gene expression clusters were identified associated with hypertrophic gene expression and mechanotransduction, extracellular matrix remodeling, inflammatory cell activation, and angiogenesis [218]. Together, these studies suggest that a coordinated transcriptional response spanning multiple cellular processes is responsible for RV adaptation to PH, and extracellular matrix-regulating mediators may play a major role in mediating RV

decompensation in PAH. Other key biological pathways identified include pro-inflammatory signaling pathways and fatty acid oxidation [217]. The second study also demonstrated sexual dimorphisms in RV adaptation, with females maintaining RV compensation longer and employing a different gene program once progressing to RV failure [217]. Targeting these pathways in a sex-specific manner may allow for targeted and personalized treatment approaches for RV failure.

In addition to translational approaches to understanding RV function at the cellular level, omics-based approaches have been applied in several settings. Metabolomic analyses of RV failure have been performed in animal models and humans. Distinct metabolomic profiles correlating with outcomes have been identified [139, 219–221], but heterogenous findings and lack of standardization of methodology mean that further validation is required. Genomic and proteomic analyses will help clarify the natural history of at-risk RVs (e.g., *BMPR2* mutation carriers, CTD, or prematurity) and RVs in non-PAH PH. Spatial transcriptomics analyses have suggested that RV adaptation to PH varies according to location within the RV [222], highlighting the locational context that spatial transcriptomics can provide to RV functional investigations. In tandem, there is an ongoing need to further phenotype and mechanistically dissect RV function using novel tools such as single cell RNA sequencing and ATAC-seq. Better definition of the contributions of sex, age, exercise, diet, and assessing time courses and changes over time is also required. Such studies should be paralleled by detailed, structural investigations of the RV to identify where most profound RV remodeling occurs so that regions of interest can be identified and defined.

6.2 | RV Hemodynamic Phenotyping

Robust RV hemodynamic phenotyping is an essential component of understanding RV adaptation and maladaptation in PH. Right heart catheterization remains key to phenotyping the RV in both research and clinical settings [1, 223, 224], and additional metrics obtained through right heart catheterization provide inference on RV response to increased loading conditions. For example, pulmonary artery pulsatility index, defined by the ratio of pulmonary artery pulse pressure to right atrial pressure, suggests RV dysfunction and clinical outcomes in other cardiac disease states, and similarly has shown value in predicting survival among patients with PAH [225], operable CTEPH [226], and inoperable CTEPH [227]. Reduced pulmonary artery pulsatility index correlates with RV sarcomere contractile dysfunction [228], suggesting a pathophysiological basis for clinically relevant hemodynamic outcomes. Along similar lines, among patients with PAH, increased ratio of right atrial pressure to PAWP predicts mortality [229]. Finally, pulmonary arterial compliance can be estimated by the ratio of stroke volume to pulmonary artery pulse pressure [16]. Decreased pulmonary arterial compliance not only correlates with PH severity, it may actually contribute to the progression of PH by inducing shear stress and distal pulmonary arterial remodeling [16]. Across subgroups of PH, decreased pulmonary arterial compliance predicts mortality [16, 40, 230].

Invasive pressure-volume analysis via conductance catheters is the gold standard method of assessing RV function [231, 232]. Pressure-volume analysis provides metrics of contractility, afterload, diastolic function, myocardial energetics, and ventricular-arterial coupling [231, 232]. Reduced ventricular-arterial coupling in patients with PAH predicts clinical worsening [233], and differences in contractility, diastolic function, and ventricular-arterial coupling reveal important physiological differences in RV adaptation across sex and PAH subgroup [234, 235]. Pressure-volume analysis can also be approximated by noninvasive imaging modalities as well as right heart catheterization pressure measurements coupled with noninvasive volumetric assessment [236]. A recent cluster analysis of metrics derived from right heart catheterization RV pressure waveforms identified distinct subphenotypes of RV function, with certain subphenotypes demonstrating decreased RV contractility and ventricular-arterial coupling [237]. Importantly, the identified RV subphenotypes contained a spectrum of patients across the traditional WSPH groups and with a range of measured mPAP and PVR [237], demonstrating the potential for advanced hemodynamic phenotyping to advance precision medicine approaches.

Finally, novel strategies of RV phenotyping via provocation, such as through exercise, are a topic of active investigation [231, 232]. RV dysfunction not apparent at rest can be unmasked with increasing cardiac output requirements during exercise [232]. Decreased RV contractile reserve during exercise predicts occult RV dysfunction and clinical worsening [238]. In a study of patients with severe idiopathic or systemic sclerosis-associated PAH, decreased RV contractile reserve during exercise predicted acute RV dilation and reduced ventricular-arterial coupling, and was associated with reduced intracellular calcium cycling [9]. Thus, exercise provocation added to hemodynamic assessment may increase the potential to comprehensively phenotype RV (mal)adaptation in pulmonary vascular disease.

6.3 | Cardiac Imaging and Radiomics: Role in Precision Medicine and Relevance for the RV

Noninvasive strategies for phenotyping the RV are beginning to provide novel insights. In seemingly stable PAH patients, cardiac imaging parameters may deteriorate before conventional biomarkers, such as functional class and 6MWD, indicating early clinical progression [239]. Volumetric assessment of the RV by cardiac magnetic resonance imaging, particularly when corrected for known differences in age and sex, supplements the prognostic value of clinical data [240, 241]. For these and other reasons, volumetric thresholds have been incorporated into the comprehensive risk assessment table in the 2022 European Society of Cardiology/European Respiratory Society Guidelines for the diagnosis and treatment of PH [1]. However, for clinicians using cardiac magnetic resonance imaging or echocardiography, cardiac imaging extends far beyond simply measuring RV volumes.

Experienced cardiac imagers have long been able to distinguish patterns in the overall shape and contraction of the RV in patients with varying disease etiologies and stages of clinical progression [242]. New image analysis tools have added to the ability to quantify the prognostic implications of cardiac

motion, including taking account of the heart as a whole, rather than focusing on one particular part of the anatomy. Machine learning of three-dimensional RV motion improves outcome prediction in PAH relative to simple volumetric assessment and standard clinical parameters [243, 244]. These techniques are only becoming reproducible now that artificial intelligence applications have improved segmentation of the cardiac chambers and muscle [245]. RV motion analysis combined with hemodynamic measurements allows for calculation of regional wall stresses, which may aid in a better understanding of the pathobiology of RV failure [246]. Other future radiomic applications of cardiac magnetic resonance imaging data include the prediction of treatment responses using T1 mapping and the association between genetic variation and patient-to-patient differences in RV adaptation [247, 248].

It is a matter of time before a combination of artificial intelligence-aided analysis of cardiac motion and the pulmonary circulation will be used in a comprehensive radiomic analysis of the cardiopulmonary unit to yield biomarkers for diagnosis, prognostication, and prediction of treatment responses [249, 250]. On the horizon are diffusion tensor imaging to study fiber orientation [251] and hydrogen, phosphorus and carbon spectroscopy to study intramyocardial lipids, energetics and metabolic flux [252]. Coupling these techniques with assessment of clinical and hemodynamic changes may allow for early identification of functional decline and patient-specific therapeutic targets.

6.4 | RV: Future Directions

RV dysfunction is the most significant predictor of mortality in PH [1] and the adaptation response of the RV rather than its afterload defines the fate of the RV [253]. Despite its prognostic value, none of the currently available PH therapies directly target the failing RV, suggesting a substantial knowledge gap regarding RV function and dysfunction. The ability to apply single cell/single nucleus RNA sequencing, advanced imaging modalities, and analytical methodology among other tools provide a robust armamentarium with which to dissect and interpret RV biology. Enhanced awareness of the importance of RV function in PH should energize new opportunities to improve health through better diagnosis, prognostication, and ultimately, therapy for RV dysfunction.

7 | Integrating Precision Medicine to Clinical Care

The new possibilities for deep phenotyping and individualized treatment in pulmonary vascular disease provided the background for dedicated discussion of the opportunities and challenges in integrating precision medicine to clinical care.

7.1 | Precision Hemodynamics

The paradigm for integrating precision medicine into clinical care is the acute response of PAH patients to a vasodilator during right heart catheterization. A ≥ 10 mmHg mPAP drop from baseline to ≤ 40 mmHg with increased or unchanged cardiac output defines a distinct phenotype which predicts a favorable response to long-term calcium channel blocker therapy with improved functional status and survival [1, 254]. Unfortunately, acute vasodilator testing is not universally employed in the work up of patients and the techniques for acute vasodilator testing are not standardized [255]. For example, vasodilator response to supplemental oxygen administered concurrently with inhaled vasodilators may be falsely interpreted as a vasodilator response, despite representing a separate phenotype without the characteristic response to calcium channel blockers [255, 256]. This is a small illustration of the work to be done to integrate knowledge into best practice.

As long as invasive hemodynamics have a role in diagnosing and phenotyping patients with PH, technical rigor is required to ensure accurate assessment [257]. Even mild elevations in mPAP and PVR among multiple underlying disease states predict reduced survival [258–262]. Furthermore, a critical component in the classification of PH is the PAWP, which may be falsely elevated in the setting of incomplete pulmonary artery occlusion [263]. PAWP determines whether a patient with PH has precapillary PH, where there are a number of treatment options, or post-capillary PH, where there are no approved treatments for PH. In addition to waveform and fluoroscopic verification, pulmonary artery occlusion may be verified through obtaining an oxygen saturation in the wedge position [263]. Technical considerations are important but even so, several common conditions including obesity, COPD, and atrial fibrillation may decrease the accuracy of end-expiration PAWP as a surrogate for LV end-diastolic pressure (typically with falsely elevated PAWP) [232, 257, 264, 265], thereby complicating the classification of PH.

7.2 | Remote Monitoring

Traditional medicine relies on intermittent and often infrequent consultations between doctor and patient. Remote monitoring offers the potential, for more frequent data collection, including gathering information on patients in their home and work environment, and may allow ‘fine tuning’ of treatment on a personal level.

Remote monitoring may consist of traditional clinic-based assessments delivered in a remote setting, such as questionnaires like the Duke Activity Status Index (a 12-point self-administered questionnaire) [266], mobile phone 6MWT applications [267], home brain natriuretic peptide kits, pulse oximeters, and even telehealth stethoscopes that capture respiratory and heart sounds. Alongside this, increasingly complex technology and algorithms are available, such as the remote dielectric sensing (ReDS) wearable vest which measures lung fluid levels (Sensible Medical Innovations Ltd) [268] and wearable devices which continuously monitor heart rate, blood pressure, cardiac rhythm, step counts, distance ambulated, oxygen saturation, and/or sleep patterns to contribute to earlier detection of decompensation [269]. Implantable devices which measure pulmonary artery pressure, such as the CardioMEMS™ HF System (Abbott) [270] and Cordella™ Pulmonary

Artery Pressure Sensor (Endotronix, Inc) [271], left atrial pressure such as the V-LAP™ system (Vectorious Medical Technologies) [272], and intrathoracic impedance as a marker of fluid overload such as the OptiVol™ 2.0 Fluid Status Monitoring system (Medtronic) [273], are also available. While many of these devices have been developed in the context of left-sided heart failure, potential applicability to pulmonary vascular disease has been demonstrated. For example, monitoring of hemodynamics in Group 2 PH via the CardioMEMS™ HF System was associated with reduced composite death and heart failure hospitalization [274]. Early data evaluating the CardioMEMS™ HF System in patients with PAH patients suggests that it similarly may be helpful in monitoring hemodynamic changes [275]. While in its nascency, remote monitoring via wearables and implantable devices offers promising opportunities to leverage emerging technologies to personalize and refine care.

7.3 | Electronic Health Records

Vast amounts of clinical, social/environmental, behavioral, and molecular data can be extracted from the electronic health record, making it a potentially cost- and time-efficient tool for research. The electronic health records of patients can be readily matched with controls. Longitudinal data can be made available and the system is self-perpetuating.

One example of the potential power of the electronic health record can be observed in its application to derive phenotype risk scores which could be coupled with genotype to discover novel associations between rare genetic variants and phenotypes [276]. A phenome wide association study was performed using the electronic health record to link genetic risk for diabetes with elevated pulmonary artery pressure and decreased echocardiographic RV-pulmonary artery coupling [277], providing new insight into shared risk for diabetes and PH which may be leveraged for novel precision medicine approaches in the future. The large-scale NIH-funded precision medicine program, *All of Us* (https://allofus.nih.gov), aims to collect genetic and health data, including from mobile/wearable technologies, from at least one million individuals with diverse backgrounds. In a recent study, *All of Us* electronic health record data was leveraged to determine that step count was inversely related to development of chronic diseases, with some diseases demonstrating a linear effect and others, like diabetes and hypertension, demonstrating no further risk reduction above 8000–9000 steps [278]. The combination of wearables with electronic health record data offers exciting possibilities to define the role of behavioral factors such as physical activity and sleep in monitoring and/or modifying diseases including PH. However, ongoing challenges to the widespread use of electronic health data exist, including the unstructured format of the electronic health record, lack of standardization of case definitions/phenotype specificity, missing data or errors, and the presence of bias [279].

7.4 | Ethics and Health Disparity

While the horizon for precision medicine in pulmonary vascular disease is bright, there are ethical issues to consider in how we apply precision medicine approaches. Specifically, many current precision medicine approaches may not be designed to benefit everyone. For example, genome-wide association studies disproportionally overrepresent individuals of European ancestry [280]. There are higher rates of variants of uncertain significance in people of non-European ancestry [281]. Multiracial individuals have largely not been accounted for in genomic research to-date [282]. Moreover, polygenic risk scores vary based on genetic ancestry and context [283], such as socioeconomic status, age, and sex [284], and their use may further exacerbate health disparities [280]. Similarly, registries used to derive phenotype data and guide design of precision medicine strategies including clinical risk scores may lack information related to social determinants of health or underrepresent minority populations [285]. Big data analysis tools without appropriate oversight may further accentuate disparities [4], whether through lack of inclusion of diverse populations in a reference dataset or by perpetuating systemic biases represented in the reference dataset. The inclusion of individuals of diverse backgrounds is critical to ongoing clinical trial design, and the application of precision medicine strategies must be thoughtful and responsible in promoting fair and unbiased strategies for diagnosis, risk stratification, and treatment in pulmonary vascular disease.

8 | Conclusion

Realizing the promise of precision medicine for pulmonary vascular disease has moved closer to practice but we are still early in the journey, particularly when compared with oncology. Omics approaches, advanced imaging techniques, and an expanding repertoire of techniques to probe molecular mechanisms provide new ways to investigate and understand biology. Powerful computational sciences approaches complement the rapidly expanding body of data, allowing for big data statistical analysis and recognition of complex patterns.

While the tools at hand create the potential for applying precision medicine to pulmonary vascular disease, successful implementation remains challenging. A vast amount of data from multi-omic and deep phenotyping approaches must be assimilated and comprehensively analyzed, all while maintaining adequate power and not losing important signals. Analytical techniques including those powered by machine learning can be employed with the goal of dimensionality reduction. However, the application of these tools and in particular artificial intelligence requires careful attention and needs to be progressive rather than disruptive. Ultimately, harnessing the power of the available tools will require data sharing, establishing standardized protocols, and robust collaboration between scientists across multiple fields, including data science. Meanwhile, the quality of the data input must be rigorously maintained, and in many cases expanded from prior efforts. In preclinical studies, multiple experimental models must be employed and coupled with careful histological, hemodynamic and functional phenotyping. For human samples and clinical research, inclusion of individuals representing a spectrum of social determinants of health as well as varying disease stages and underlying PH pathophysiology will result in a more representative depiction of the disease.

The challenges are significant, but so too are the opportunities. Through careful and responsible implementation of advanced technology upon a framework of scientific rigor, we will approach the asymptote of precision medicine in pulmonary vascular disease.

2023 Grover Conference Series

This review article is part of the 2023 Grover Conference Series. The Grover Conference is named in recognition of the significant contributions of Dr. Robert Grover to the fields of high-altitude medicine and pulmonary vascular disease. Dr. Grover's protégé, Dr. John "Jack" Reeves, developed the concept for the conference series and was instrumental in its organization. The inaugural Grover Conference was held in 1984 in Sedalia, Colorado. The 2023 Grover Conference was held at The Devil's Thumb Ranch in Tabernash, Colorado. The resort had many modern amenities and activities such as hiking and horseback riding. The organization of the 2023 Grover Conference program was led by Drs. Anna Hemnes, Vinicio de Jesus Perez, and Martin Wilkins. There were 57 speakers/session chairs and 78 attendees. Memorial lectures were given in honor of significant Grover Conference Series contributors, including John "Jack" T. Reeves (Paul M. Hassoun), Robyn J. Barst (Kara N. Goss), Estelle B. Grover (Stephen Y. Chan), and Terry Wagner (Kurt R. Stenmark).

A notable conference participant was Dr. E. Kenneth Weir. Dr. Weir has attended all of the Grover Conferences since its inception. He was director of each of the first ten Grover meetings from 1984 to 2000. Moreover, as his sweater is made of high-quality wool, he has worn this same sweater at many Grover conferences, from the inaugural Grover Conference to the present one (Figure 4). Dr. Weir shared the following poem in honor of his close friend and Grover Conference founder, Dr. John "Jack" Reeves [286].

For J T R

The sun rose in Hazard.

For many years he towered in our sky,

warm, strong and nurturing,

sometimes fierce and scorching.

Uncompromising,

FIGURE 4 | Top left: Drs. Jack Reeves, future Nobel Laureate Robert Furchgott, and E. Kenneth Weir at the inaugural 1984 Grover Conference [287] Top right: Drs. Weir, Kurt Prins, Sasha Prisco, and Thenappan Thenappan at the 2023 Grover Conference. Bottom: Conference participants at the 2023 Grover Conference.

like an Old Testament prophet,

his passion etched in the lives of others.

Now, suddenly, the light is gone

as when the sun drops behind the Colorado Rockies.

But his warmth stays in our hearts

and the light which burned in his eyes

must shine in ours.

John T Reeves MD. "Jack," was born in Hazard, Kentucky, 11/17/1928. He was an internationally respected scientist and a beloved mentor to many at the Cardiovascular Pulmonary Laboratory of the University of Colorado in Denver. He died in Colorado, 9/15/2004.

Author Contributions

All authors contributed to drafting, review, and final approval of the work. Lindsay M. Forbes is the guarantor of the work.

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Conflicts of Interest

ARH has served as a consultant for United Therapeutics, Bayer, GossamerBio, Merck, Janssen and Tenax Therapeutics and has stock in Tenax Therapeutics. AJ has previously served on an advisory board for Janssen (12/2023. GAH receives funding from Bayer Healthcare: non-branded Speakers Bureau, Advisory Boards, and Research Funding; and Janssen Pharmaceuticals: Clinical Trial Steering Committee and Advisory Boards.

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