




Precision medicine and personalising therapy in pulmonary hypertension: seeing the light from the dawn of a new era

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ABSTRACT Pulmonary hypertension (PH) and pulmonary arterial hypertension (PAH) include different cardiopulmonary disorders in which the interaction of multiple genes with environmental and behavioural factors modulates the onset and the progression of these severe conditions. Although the development of therapeutic agents that modulate abnormalities in three major pathobiological pathways for PAH has revolutionised our approach to the treatment of PAH, the long-term survival rate remains unsatisfactory. Accumulating evidence has underlined that clinical outcomes and responses to therapy in PAH are modified by multiple factors, including genetic variations, which will be different for each individual. Since precision medicine, also known as stratified medicine or personalised medicine, aims to better target intervention to the individual while maximising benefit and minimising harm, it has significant potential advantages. This article aims to assemble and discuss the different initiatives that are currently underway in the PH/PAH fields together with the opportunities and prospects for their use in the near future.

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Introduction

Pulmonary hypertension (PH) refers to a haemodynamic and pathophysiological condition that is defined as a persistent elevation of the mean pulmonary arterial pressure ≥ 25 mmHg at rest confirmed by right heart catheterisation, which leads to right ventricular failure and premature death. The symptoms of PH are nonspecific and include breathlessness, fatigue, weakness, angina and syncope and take years to appear, usually after the PH has reached an advanced stage. PH can result from pre-capillary (arterial) or post-capillary (venous) pathogenic mechanisms and occurs in a large group of diseases [1, 2].

Since the second world symposium on PH held in Evian in 1998, a clinical classification was established using a precision medicine approach in order to individualise and gather together different categories of PH sharing similar haemodynamic criteria, types of pulmonary vascular lesions and similar management [1, 2]. Indeed, the current clinical classification for PH attempts to reconcile pathological findings with new pathobiological concepts and separates conditions with a predominantly pulmonary arterial disease component (group 1, also known as pulmonary arterial hypertension (PAH)) from four other disease categories composed of PH associated with left heart disease (group 2); PH associated with lung diseases and hypoxia (group 3); PH due to chronic thromboembolism (group 4); and PH due to unclear multifactorial mechanisms (group 5).

This clinical PH classification tool is well accepted and widely used in the daily practice of PH experts, allowing not only a more rapid introduction of targeted treatment avoiding harm and increasing benefit, but also facilitating outcome prediction and research strategies. However, this “one size fits all” approach yields an average treatment effect for the average patient, but is suboptimal for choosing therapy at the individual patient level. Within a clinical trial, there are probably certain groups of patients who respond very well to a treatment and groups of patients who respond poorly or who are made worse. The next step would be to include biomarkers, proteomics and genomics to further develop precision medicine strategies leading to personalised medicine for PH patients; however, several challenges remain. The objective of this review is to summarise some of the most common challenges and provides a brief overview on the existing literature.

How could precision medicine add to current PAH/PH management?

The goal of personalised medicine is to streamline clinical decision to provide the right treatment strategy for a given patient based on his or her individual characteristics (figure 1) [3]. It consists of classifying each patient into a subpopulation, taking into account their susceptibility to develop a particular disease or phenotype and its response to a treatment. Precision medicine, which is more ambitious, encompasses both the treatment and the prevention of the disease based on individual genetic, environmental and lifestyle differences. This new approach depends on the availability of molecular profiling tests for a particular clinical condition. The development of targeted therapies and immunotherapy in the treatment of cancer demonstrates that precision medicine is realisable. This new approach has allowed the possibility of more rapid introduction of cancer drugs and treatments according to a cancer’s molecular profile regardless of location or histological analysis.

For patients with idiopathic/heritable PAH, the 5- and 7-year survival rates from time of diagnostic right-sided heart catheterisation are 57% and 49%, respectively, despite currently available medical treatment advances [4]. PAH is not a cancer, but similarities can be observed concerning the altered crosstalk between cells from different tissue types, unexplained proliferation and survival of pulmonary smooth muscle and endothelial cells, the metabolic (glycolytic) shifts and the association with the immune system [5]. PAH is characterised by vasoconstriction, cell accumulation in the vascular wall and intimal thickening of the small- to medium-sized pulmonary arteries ($\leq 500 \mu\text{m}$) in the absence of major pulmonary parenchymal changes or embolic events [5–9]. Current PAH-specific therapies mainly induce relaxation of the pulmonary arteries. In a small group of PAH patients that respond to acute vasodilators, relaxation of pulmonary vascular smooth muscle can be achieved with calcium channel blockers. However, most patients are treated with specific medication approved by the United States Food and Drug Administration (US FDA) to modulate abnormalities in three major pathobiological pathways for PAH: the nitric oxide, prostacyclin and endothelin pathways [2]. Lung transplantation is the last therapeutic option if the disease progresses or medications fail.

Although different forms of PAH/PH could reflect distinct pathophysiological mechanisms, current evidence strongly suggests that a common denominator underlying many of the established molecular and cellular elements exists [5–9]. Indeed, recent discoveries support the concept that this irreversible pulmonary vascular remodelling in PAH results from two complementary mechanisms: inherent characteristics of resident pulmonary vascular cells, including pulmonary artery smooth muscle cells (SMCs), myofibroblasts, endothelial cells and pericytes; and dysregulation of molecular events that govern pulmonary vascular cell growth/survival, including signals originating from microenvironmental alterations in the pulmonary arterial wall. Although tremendous advances have been made, the

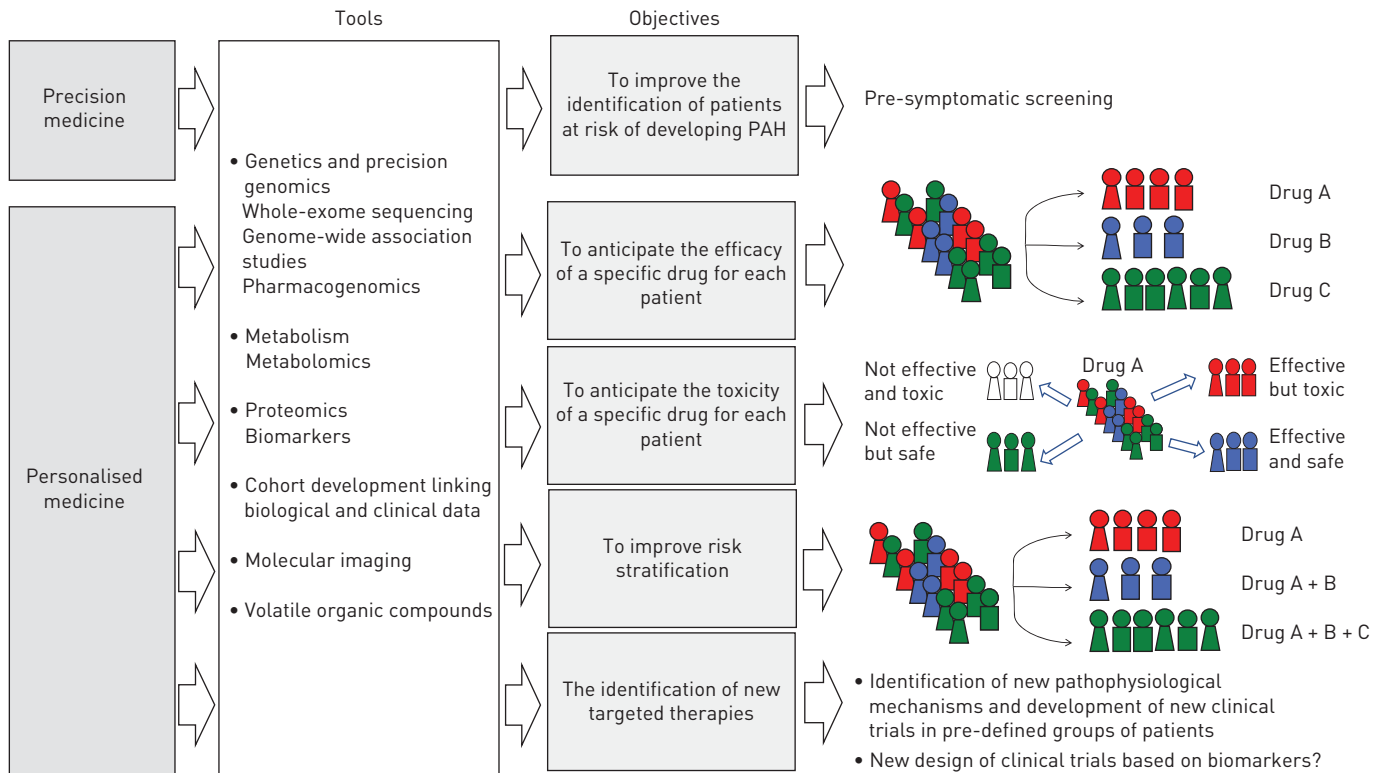


FIGURE 1 New “-omics” approach for personalised medicine. PAH: pulmonary arterial hypertension. Reproduced and modified from [3] with permission.

pathobiology remains poorly understood. Furthermore, it is now well established that the presence of genetic or environmental modifying factors can influence the onset and the progression of these severe cardiopulmonary disorders.

When considering idiopathic PAH patients, we are now beginning to appreciate a high degree of phenotypic variability within this subgroup. In the historical cohort from the National Institute of Health, patients with idiopathic PAH were young (mean age 31 years) with a female predominance [10]. Since then, idiopathic PAH has been increasingly diagnosed in older patients with a median age at diagnosis that continues to climb in national and international registries. A significant proportion of patients now present with other comorbidities that may influence prognosis, progression of disease and, probably, response to treatment [11, 12]. Current guideline recommendations only take into account the clinical classification of PH and disease severity at diagnosis and during follow-up, resulting in highly variable treatment responses from one individual to the next. Therefore, it seems necessary to develop better methods of predicting responses to each therapeutic class of medication and to anticipate disease evolution at the individual level to optimise prognosis through individualised therapeutic choices. In addition, this high degree of phenotypic variability between the different PH entities or even within the same subgroup is a source of patient heterogeneity in clinical trials.

New “-omics” approaches permit the acquisition of large amounts of data at multiple biological levels, from gene sequencing to protein expression and metabolic profiling. Such data can cover all potential mechanisms implicated in underlying cellular networks that influence the function of organ systems in their totality [13]. This inversion of the classical research model (from traditional experimentation based on precise hypotheses to a model of research that does not depend at all on such hypotheses) opens the door in an unprecedented way to many new discoveries on the pathophysiological mechanisms in PAH, as well as to a better understanding of the molecular factors that influence the efficacy of existing therapies and prognosis. Therefore, our current approach must evolve urgently. The new era will be one not based on pre-specified hypotheses stemming from animal models, but one based on the identification of new pathophysiological mechanisms that are actually implicated in PAH in humans, using large-scale analyses of biological, molecular and genetic variations associated with the development of PAH. This new approach necessitates a reorganisation of research methods. Henceforth, it will be essential to establish biobanks from cohorts included in registries or clinical trials and to develop tissue banks from explanted lungs from patients who undergo lung transplantation. These new research perspectives will allow

comparisons of patient characteristics, prognosis and responses to different classes of medications according to their epigenetic, genetic, proteomic and metabolomic signatures. We can hope that, sooner rather than later, clinicians will be able to establish a sort of individual “-omics map” to accurately anticipate the risk of progression and choose the most appropriate and effective therapy for each patient and improve outcomes. Importantly, the use of clinical phenotype and classification tools could help to minimise patient heterogeneity in PH clinical trials.

What types of molecular testing could be used?

Considerable progress has been achieved in oncology through the comprehensive assessment of the molecular features of patients and their therapeutic needs. In addition to the availability of clinical trials or compassionate use of specific therapeutic agents, these achievements typically relied on assays of blood and tissue that allow access to tumour specimen characterisation, well-validated circulating biomarkers and molecular imaging, among others. In PAH, tissue access is a clear limitation, since lung biopsy carries prohibitive risks in most PAH patients. Furthermore, the recent emergence and application of a large number of other new technologies in the field may lead to a precision medicine paradigm in the near future.

Genomics approaches have successfully identified several genes and genetic loci involved in the predisposition or in the development of PH/PAH [14–41]. These findings have revealed complex multifactorial aspects of the PH/PAH pathogenesis and have provided insights for disease diagnosis, treatment and prevention. Additionally, other omics-based technologies have been used in preclinical research to evaluate changes in other biomolecules, such as epigenomics for epigenetic markers, transcriptomics for mRNAs, non-coding RNAs and small RNAs, proteomics for proteins and peptides and metabolomics for low molecular weight metabolites. The utility of molecular classification to support clinical classification has been recently developed [42–44] and should foster the use of these technologies in the future. Indeed, HEMNES *et al.* [43] identified several rare gene variants involved in the acute vasodilator response. Another example of how pharmacogenomics can be used to personalise PAH treatment with currently available drugs comes from BENZA *et al.* [45] who used genome-wide association studies to identify single-nucleotide polymorphisms in the endothelin-1 gene, endothelin receptor A and B genes and G-protein subunit genes. They identified a variant in the *GNG2* gene on chromosome 24 that was associated with better response to endothelin receptor antagonist therapy. Identification of favourable and detrimental polymorphisms in other PAH pathways, including the nitric oxide and prostacyclin pathways, may improve efficiency and selection of patients for future studies of existing or novel PAH therapies, including gene editing, gene replacement and endothelial progenitor cell therapies [46, 47].

There is a clear need to identify well-validated circulating or molecular imaging biomarkers in PH/PAH. In this context, RHODES *et al.* [44] performed a proteomic analysis in 143 consecutive patients with idiopathic and heritable PAH with 2 years of follow-up and in a further 75 patients with 2.5 years of follow-up. They identified that the measurements of a combination of nine circulating proteins can be used to stratify PAH patients with a high risk of mortality, independent of existing clinical assessments, which might have a use in clinical management and the evaluation of new therapies. This panel of nine prognostic proteins has been identified using an aptamer-based assay of 1129 plasma proteins and includes interleukin-1 receptor-like 1, tissue inhibitors of metalloproteinases-1 and -2, plasminogen, apolipoprotein-E, erythropoietin, complement factors H and D and insulin-like growth factor binding protein-1. Interestingly, the functions of these proteins were related to myocardial stress, inflammation, pulmonary vascular cellular dysfunction and structural dysregulation, iron status and coagulation. In addition to genomics and metabolomics, these circulating biomarkers could clearly help to obtain a better comprehensive assessment of the molecular features of PH/PAH patients and therefore needs to be validated.

Other techniques are in development with the objective of screening and early detection of PAH at the individual level. For example, volatile organic compounds (VOCs) have been proposed as noninvasive biomarkers for PAH and an “artificial nose” has been developed with the ability to analyse the pattern of exhaled VOCs unique to an individual. Pathological processes affect the production and may drive specific profiles of VOCs in the exhaled breath, known as the “exhaled volatolome”. In this way, the identification and quantification of an exhaled volatolome could provide important, personalised, noninvasive biomarker information [48].

POLLETT *et al.* [49] reported a new technique to harvest pulmonary arterial endothelial cells (PAECs) from discarded Swan–Ganz catheter balloons after routine right heart catheterisation. The major advancement of this new technology is the ability to isolate PAECs from a living human rather than from explanted or *post mortem* tissue. This approach could have the potential for future applications to test pharmaceutical responses *in vitro* for individual patients and to provide a direct examination of changes in endothelial

biology during disease progression and in response to treatment [50, 51]. The use of induced pluripotent stem cell (iPSCs) to derive endothelial or smooth muscle cells from PAH patient could represent an alternative and a path to move PAH treatment into personalised medicine [52, 53].

Is precision medicine ready for use in PAH/PH?

The practice of precision medicine is not really new in PH/PAH. For decades, pulmonary vasodilator testing to inhaled nitric oxide has been used to guide management of patients with PAH. Patients meeting vasoreactive criteria during right heart catheterisation are known to have favourable long-term responses to oral calcium channel blockers and better survival [54, 55]. Although vasodilator-responsive PAH patients are a minority (<10%) of idiopathic PAH patients, recent studies have found distinct genetic and molecular pathways in vasodilator-responsive patients compared to nonvasoresponsive PAH [43, 56], supporting the utility of molecular techniques in predicting drug responsiveness in PH/PAH.

While precision medicine has the potential to profoundly improve the PH/PAH management, different challenges must be resolved to extend precision medicine approaches into mainstream practice. First, a better understanding of how altered immune responses may contribute to PAH initiation, perpetuation and worsening is needed. It is now well established that altered inflammatory and immune mechanisms play a significant role in PH/PAH by recruiting inflammatory cells, remodelling the pulmonary vasculature and promoting autoimmune responses. Indeed, there is complex interplay between PAH and autoimmune disorders (such as systemic lupus erythematosus, Sjögren's syndrome, scleroderma and thyroiditis) or chronic infections (HIV, bilharzia and human herpes virus type 8). Some of these clinical presentations may benefit from specific management, leading to a distinct outcome from idiopathic PAH. For example, pulmonary vascular disease may be reversible with immunosuppressant medications in some patients with lupus or mixed connective tissue disease-associated PAH, whereas this type of response to immunosuppressive medications is not observed in systemic sclerosis-associated PAH [57]. An unusual response to specific PAH therapies (including endothelin receptor antagonists) has also been observed in particular forms of PAH that are associated with immune disorders and an inflammatory component that probably plays a crucial role. Thus, cases of normalisation or quasi-normalisation of resting haemodynamics have been reported in patients with HIV or cirrhosis [58, 59]. More generally, inflammation and immune disorders appear to be common denominators for all forms of PAH. In PAH, pulmonary vascular lesions are characterised by varying degrees of perivascular inflammatory infiltrates, comprising of T- and B-lymphocytes, macrophages, dendritic cells and mast cells. Furthermore, PAH is associated with increased circulating and lung levels of certain cytokines, chemokines and adipokines [8]. Interestingly, some of these circulating inflammatory mediators correlate with a worse clinical outcome in PAH patients [60, 61]. In addition, circulating anti-endothelial cells and antifibroblast antibodies are found in 10–40% of patients suffering from idiopathic PAH [62–65], which may indicate the presence of autoimmune mechanisms linked to complement activation in the genesis of pulmonary vascular lesions of PAH. Consistently, altered regulatory T-cell function [66, 67], T-helper 17 cell immune polarisation [68], dendritic cell recruitment in pulmonary vascular lesions [69] and the presence of lymphoid neogenesis in lungs have been demonstrated in patients with PAH [70, 71]. However, the fact that steroid or aspirin treatment are clearly not effective in idiopathic and heritable PAH [72], and that prostacyclin, which has anti-inflammatory properties [73–75], does not reverse the pulmonary vascular remodelling and PAH, support the need for a better characterisation of the involvement and mechanism of distinct immune cells and key inflammatory mediators. This better knowledge could pave the way to novel therapeutic strategies for predefined groups of patients and could help to identify panels of key inflammatory mediators to distinguish different PH/PAH clinical phenotypes.

Second, the reasons why bone morphogenetic protein receptor (BMPR)-II signalling is reduced in PAH and how the alterations influence or even drive the pathogenesis need to be understood. Indeed, heterozygous *BMPR2* germline mutations account for more than 75–80% of familial PAH and ~15–20% of sporadic cases. However, the average penetrance of *BMPR2* pathogenic variants is low and the lifetime risk of developing PAH with a *BMPR2* pathogenic variation in a male is 14%, whereas in a female it is 42% [38]. Because there is evidence that *BMPR2* mutation carriers have a worse prognosis [38], are less likely to respond acutely to vasodilators and have a relatively preserved diffusing capacity of the lung for carbon monoxide compared to non-carriers [76, 77], a systematic screening for the presence of this predisposing mutation is recommended. Evidence suggests that the degree of vascular remodelling is greater in lungs of patients with *BMPR2* mutations compared with lungs of non-*BMPR2* related disease at the time of transplantation [78, 79]. Interestingly, for an unknown reason, BMPR-II or its downstream signalling pathways are reduced to a similar extent in explanted lungs of non-carriers, highlighting the need of further studies to identify the underlying mechanisms [80]. Additional mutations in several other genes (>1% of families with PAH) have been identified, namely *ACVRL1* (activin receptor-like kinase (ALK-1)), *BMPR1B* (ALK-6), *GDF2* (bone morphogenetic protein-9), *TBX4* (T-box 4), *ENG* (endoglin),

SMAD9 (Smad-8), *CAV* (caveolin) and *KCNK3* [81]. The development of genetic counselling and the analysis of the phenotypic profile of patients according to their genotype have helped to develop preventive medicine and a specific monitoring for *BMPR2* mutation carriers. Therefore, pre-symptomatic screening of individuals with known mutations in PAH-predisposing genes could potentially lead to earlier diagnosis and treatment of PAH. Considering the above, the 2015 European Society of Cardiology/European Respiratory Society guidelines currently recommend that “individuals who test positive for PAH-causing mutations and first degree relatives of heritable PAH cases may be considered to have an annual screening echocardiogram (IIB-C)”. The clinical presentation of heritable PAH at diagnosis and its evolution can be influenced by the identified genetic mutation leading in some cases to a personalisation of therapeutic choices and monitoring. Patients with established PAH and a *BMPR2* mutation have an increased risk of death or transplantation [38]. In addition, biallelic *EIF2AK4* mutations, known to associate with pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis have been recently discovered [82] allowing appropriate management and early referral for lung transplantation.

Thus, not all carriers of a *BMPR2* mutation will develop PAH, suggesting that other factors, genetic and/or environmental, are required to induce PAH. While causal in disease pathogenesis, the exact mechanism between BMPR-II dysfunction and the development of PAH is not known. Even in the absence of a mutation, BMPR-II expression is reduced in PAH [83]; therefore, improving BMPR-II signalling could be applicable beyond those with heritable or sporadic *BMPR2* mutations. A genomics-driven classification with therapeutic strategies targeting the BMPR-II pathway is an attractive paradigm for precision medicine in PAH patients.

Gene editing and gene therapy are promising emerging technologies that could correct pathogenic mutations in patients with heritable PAH [3]. Using an adenovirus vector, REYNOLDS *et al.* [84] showed that *BMPR2* gene delivery could restore BMPR-II expression, improve haemodynamics and attenuate vascular remodelling in monocrotaline-induced and chronic-hypoxia models of PH in rats. More recent studies have confirmed the ability of *BMPR2* gene therapy to improve PAH in animal models by increasing Smad1/5/8 signalling and possibly by increasing nitric oxide production in human pulmonary endothelial cells [85]. YAN *et al.* [86] showed that myeloablative radiation followed by transplantation of donor stem cells from mice with normal *BMPR2* into mice with *BMPR2* mutations prevented the development of PH. Although these pre-clinical data are promising, there are likely to be significant challenges in translating *BMPR2* gene therapy to humans with our current limitations in vector delivery to the pulmonary endothelium, immunogenicity and uncertainty regarding whether increasing BMPR-II expression results in sustainable long-term improvements in PAH.

Other methods of restoring BMPR-II expression and function have been sought. SPIEKERKOETTER *et al.* [87] used a high-throughput transcriptional assay to screen 3756 compounds that were already approved by the US FDA for BMPR-II activation. They found that tacrolimus (FK506), a calcineurin inhibitor commonly used as an immunosuppressant, activates the BMPR-II pathway even in the absence of functional BMPR-II receptor. In addition, tacrolimus prevented and even reversed PH in rodent monocrotaline and Sugen-hypoxia models. In a subsequent phase IIa study, they reported that low-dose FK506 improved BMPR-II signalling in three patients with end-stage PAH without *BMPR2* mutations, with concurrent clinical improvement [88]. In a recent double-blind placebo-controlled randomised study, SPIEKERKOETTER *et al.* [89] used low dose FK506 in New York Heart Association functional class II and III PAH patients. In the 23 patients randomised, BMPR-II expression was compared to healthy controls and FK506 was well tolerated overall; however, there were no significant improvements in clinical variables such as 6-min walking distance, N-terminal pro-brain natriuretic peptide, echocardiographic measures of right ventricular function or BMPR-II expression compared to placebo [89]. There have been no studies or reports of FK506 used in patients with heritable PAH due to *BMPR2* mutations, specifically. Two other pre-clinical studies have shown that chloroquine can improve BMPR-II signalling by inhibiting lysosomal degradation of BMPR-II, which prevents proliferation of pulmonary artery SMCs and progression of PH [90, 91].

Finally, other challenges such as data quality/integrity, reproducibility, security and study sample sizes have to be addressed. The small number of multi-omics datasets and the lack of standardised and harmonised protocols is also a limitation that affects the dissemination of these approaches.

Conclusion

Enthusiasm for the prospect of precision medicine has grown significantly in the last few years. However, the PH/PAH field already has several emerging clinically actionable tools useful for precision medicine and even if the translation of these technologies into clinically tools is slow, their usages will probably increase in the near future.

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