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Drug Development and Clinical Trials

Program Description

The mission of *Advances in Pulmonary Hypertension* is to serve as the premiere forum for state-of-the-art information regarding diagnosis, pathophysiology, and treatment of pulmonary hypertension (PH). The 2018 Nice revision of the World Symposium on Pulmonary Hypertension (Simmonneu G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1). DOI:10.1183/13993003.01913-2018) serves as a guide to categories of PH addressed in *Advances in Pulmonary Hypertension*. While focusing on Group 1 PH (PAH: pulmonary arterial hypertension), the other categories (Group 2, PH due to left heart disease; Group 3, PH due to lung diseases and/or hypoxia; Group 4, PH due to pulmonary artery obstructions; Group 5, PH with unclear and/or multifactorial mechanisms) are also addressed. This mission is achieved by a combination of invited review articles, roundtable discussions with panels consisting of international experts in PH, and original contributions.

Objectives

- Provide up-to-date information regarding diagnosis, pathophysiology, and treatment of PH.
- Serve as a forum for presentation and discussion of important issues in the field, including new paradigms of disease understanding and investigational trial design.

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Advances in Pulmonary Hypertension: Official Journal of the Pulmonary Hypertension Association is a quarterly publication directed by an editorial board of renowned pulmonary hypertension (PH) experts with oversight by PHA's Scientific Leadership Council. The mission of *Advances in PH* is to assist physicians in their clinical decision-making by informing them of important trends affecting their practice and providing an analysis of the impact of new findings and current information in peer-reviewed publications. Each article is reviewed and approved by members of the Editorial Board.

While most articles are invited by the Editorial Board, the following submissions will be considered for publication:

- Reviews that summarize and synthesize peer-reviewed literature to date on relevant topics
- Letters to the Editor
- Clinical case studies

Submitted manuscripts are reviewed by the Editorial Board and other experts in the field. Acceptance of manuscripts is determined by factors such as quality, relevance, and perceived value to clinical decision-making.

***Advances in Pulmonary Hypertension*'s Web Platform**

*Advances in Pulmonary Hypertensio*n is available at www.AdvancesinPH.org. This site offers enhanced capabilities including a dedicated DOI and cross-referencing potential, as well as other features valuable to practitioners, researchers, and educators. Registration is recommended to access all site features and benefits.

It is a great pleasure to introduce this issue's Guest Editors of *Advances in Pulmonary Hypertension.* Drs Mark Nicolls and Marc Humbert lead an elite group of experts in a timely compilation on drug development and clinical trials in pulmonary hypertension (PH).

The opening article by Drs Martin Wilkins and Marlene Rabinovitch eloquently illustrates novel exciting targets for drug development. These targets are based on the understanding of the mechanisms driving pulmonary arterial hypertension (PAH). They discuss this "mechanism-driven" focused approach to developing new therapies. The authors detail many of these targets and where they are in the pathway of development.

Dr Tsukasa Shimauchi and colleagues follow with an important discussion of the challenges and limitations in the design of preclinical PH trials. They emphasize the need for the rigorous assessment of study design from each part of the development, including researchers, peer reviewers, funding agencies, and academic institutions, among others.

This is followed by the expert roundtable discussion focused on the topics of drug development, clinical trials, and the importance of publishing of negative trial results. Drs Marc Humbert, Mark Nicolls, Norman Stockbridge, and Roham Zamanian examine the importance and the opportunities that a negative clinical trial provides to all of us as a community.

In our "Ask the Expert" column, Drs Christine Garnett and Norman Stockbridge, both experts on trial regulation from the US Food and Drug Administration, discuss the challenges of finding new therapies with a novel mechanism of action, of improving the efficiency of clinical trials, and of developing endpoints that reflect benefits in patient symptoms and quality of life.

We conclude this issue with a poignant discussion by Lena Bolivar. Lena, a patient who was diagnosed with PAH in 2011, takes us through her experience, starting with diagnosis of PAH, initiating therapy, going through a clinical trial, and ultimately receiving a heart-lung transplant. It is because of Lena and all of our patients that we as a community continue to work together to define new targets and develop improved therapies for the present and the future.

Congratulations and thank you, Drs Nichols and Humbert, for an exceptional issue.

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GUEST EDITOR'S MEMO

This issue of *Advances in Pulmonary Hypertension* addresses the challenges and opportunities of drug development for pulmonary arterial hypertension (PAH) patients. As combinational vasodilator regimens continue to improve the lives of PAH patients, there is growing interest in also targeting novel pathways implicated in disease pathogenesis. To this end, Drs Martin Wilkins and Marlene Rabinovitch summarize exciting new disease pathways which show promise as therapeutic targets. Enthusiasm for these emerging therapies are linked to high-impact preclinical publications. In a review that highlights the importance of experimental rigor, Dr Tsukasa Shimauchi and colleagues make a compelling call for strong study design to optimize the likelihood of subsequent successful clinical trials. However, even trials that fail to show benefit for a drug can educate the PAH community. In a wide-ranging roundtable discussion, we moderate a discussion with Drs Roham Zamanian and Norman Stockbridge to address the merits of publishing negative trials, as well as the current obstacles for developing new drug treatments. Lena Bolivar, a PAH patient, then describes what it's like to participate in a clinical trial. Finally, this compendium of articles concludes with a perspective written by two US Food and Drug Administration leaders who discuss strategies for improving trial design, primary endpoints, disease biomarkers, and pediatric care.

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Novel and Relevant Mechanistic Pathways

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

INTRODUCTION

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

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

TARGETS SUPPORTED BY GENETICS

Bone Morphogenetic Protein Signaling

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

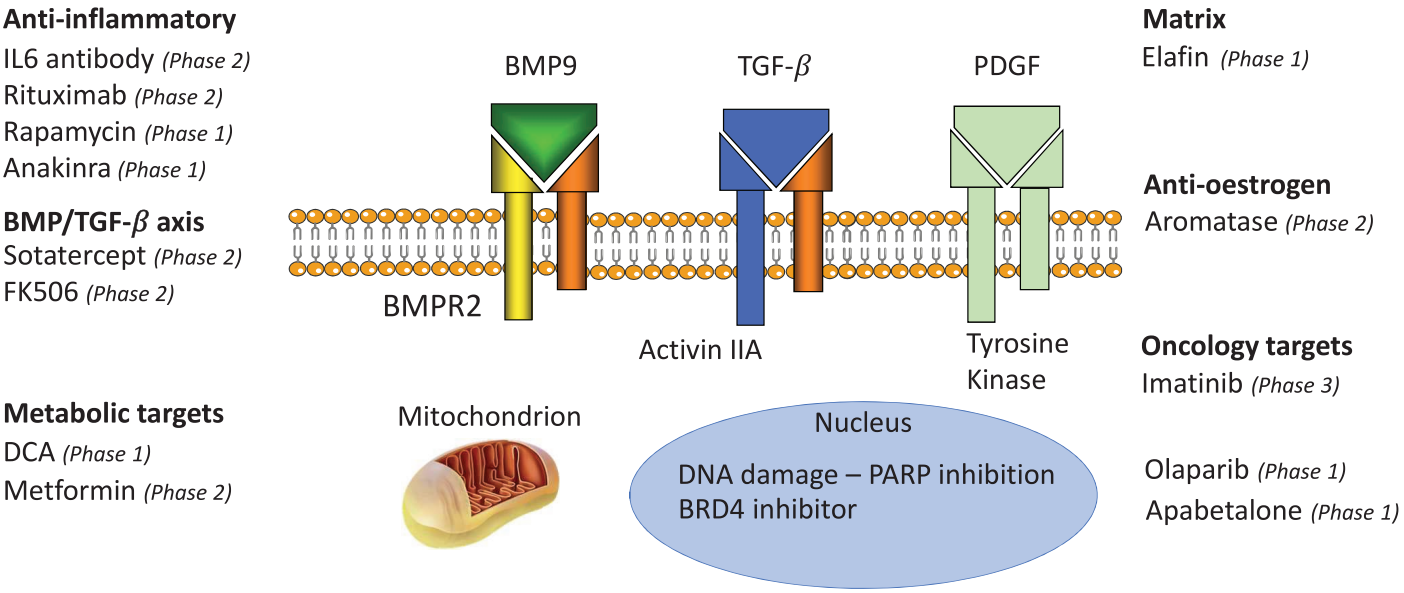

Figure 1: Selected mechanism-based novel therapeutics that have undergone or are planned for human studies. BMPR indicates bone morphogenic protein receptor; BRD, bromodomain; DCA, dichloroacetate; PDGF, platelet-derived growth factor; PARP, polyADP-ribose polymerase; TGF, transforming growth factor.

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

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

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

Other strategies for increasing BMPR-2 signaling are directed at suppressing nonsense mutations, using chemical chaperones (eg, 4-phenyl hydroxybutyrate), or inhibiting lysosomal degradation (eg, using hydroxychloroquine) to increase cell surface expression of BMPR-2.[7] Loss-of-function variants in *GDF2*, which encodes BMP9, a ligand for BMPR-2, underscore interest in the development of BMP9 as a therapy for PAH.[8] Preclinical studies with BMP9, structurally altered to reduce ectopic bone formation, are well advanced.

An alternative approach, namely, dampening exaggerated activity of TGF-β receptor signaling, is to inhibit the activin IIA receptor using a ligand trap. Sotatercept, developed to treat myelodysplastic syndromes and anemia, has shown efficacy in abrogating pulmonary hypertension in the Sugen 5416–hypoxia rat and mouse models and in the monocrotaline rat model.[9] A Phase 2 trial for PAH has shown significant reduction in the primary endpoint of pulmonary vascular resistance and also in secondary endpoints such as 6-minute-walk distance and N-terminal pro b-type natriuretic peptide as reported in early press releases (ClinicalTrials.gov Identifier: NCT03496207).

Hypoxia-Inducible Signaling Pathway

The Tibetan genome contains variants in *EPAS1*, encoding hypoxia-inducible (HIF)2α, and *EGLN1*, encoding prolyl hydroxylase 2, that may contribute to physiological adaptation to a hypoxic environment, for example, their relative resistance to hypoxia-induced pulmonary hypertension. The assumption is that the variants in *EPAS1* lead to loss of function in HIF2α while those in *EGLN1* are associated with gain of function in prolyl hydroxylase 2.[10] Subjects harboring a genetic mutation leading to HIF2α overexpression show evidence of pulmonary hypertension.[11,12] Dissociating the effect of these genotypes on hematocrit from a direct effect on pulmonary vascular homeostasis is difficult; a rise in hematocrit increases blood viscosity, which adversely affects pulmonary artery pressure. But genetic manipulation of HIF signaling in rodents suggests that focusing on HIF2α, which is expressed predominantly in vascular endothelial cells, has promise.

As a transcription factor, HIF2α is a challenge for small-molecule inhibition, but targeting the hydrophobic cavity in the inner core of the per-ARNT-Sim-B domain offers the opportunity to allosterically disrupt its dimerization to aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIFβ).[13] A series of highly selective, orally bioavailable HIF2α inhibitors have now been developed for exploration in oncology and PT2567 has shown efficacy in rodent models of pulmonary hypertension.[14]

Rare Variants in the Human Genome

Whole-genome sequencing has provided a valuable insight into the genetic architecture of PAH. Studies to date have revealed or confirmed rare variants in 16 genes.[15] A number, such as *KCNK3*, *TBX4*, *SOX17*, *ATP13A3*, *AQP1*, and *ABCC8*, lie outside the TGF-β signaling pathway, highlighting molecular heterogeneity in PAH. The extent to which these genetically defined targets are druggable remains to be determined.

TARGETS "BORROWED" FROM ONCOLOGY

Pyruvate Dehydrogenase Kinase

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

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

Tyrosine Kinases

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

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

Poly-ADP-Ribose Polymerase 1

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

Forkhead Box O1

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

Histone Deacetylase and Bromodomain-4

There are 18 histone deacetylases (HDACs) grouped into 4 classes. Inhibition of class I and class II have been explored in cell and animal models, based in part on the increased expression of HDAC1 and HDAC5 in the lungs of patients with PAH.[23] Concerns about off-target effects, particularly adverse effects on the myocardium, have delayed studies in humans and prompted the search for isoform-selective HDAC inhibitors, in the expectation of a better safety profile.

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

TARGETS SUGGESTED BY EPIDEMIOLOGY

Aromatase

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

Aromatase is produced in pulmonary arteries in both female animal models of pulmonary hypertension and in women with PAH.[27] Support for targeting aromatase comes from studies showing that a single-nucleotide polymorphism in the promoter region of aromatase is associated with a higher level of E2 and increases the risk of PAH in patients with cirrhosis.[28] Administration of the aromatase inhibitor anastrozole reduced pulmonary arterial pressures, pulmonary vascular changes, and indexes of right ventricle hypertrophy in experimental pulmonary hypertension.[27] Of note, metformin has similar effects via aromatase inhibition.[29] In a proof-of-concept clinical trial, anastrozole significantly reduced E2 levels in patients with PAH.[30] It was safe, well tolerated, and improved 6-minute-walk distance but there was interindividual patient heterogeneity in response. It remains to be established whether aromatase inhibition is the optimal approach to inhibiting estrogen. Dual inhibition with fluvestrant has been reported to be more effective in animal models and tamoxifen has been suggested for premenopausal women.[31]

Insulin Resistance

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

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

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

TARGETS IDENTIFIED FROM THE VASCULAR PATHOLOGY

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

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

A novel approach is to inhibit neutrophil elastase. Elevated levels of circulating elastase are attributable to activation of neutrophils and the formation of damaging neutrophil extracellular traps.[39] Heightened activity of this enzyme leads to breakdown and loss of elastin and the chemoattractant properties of elastin degradation products or peptides perpetuates inflammation. Inhibition of neutrophil elastase reverses experimental pulmonary hypertension in a rat model induced by monocrotaline.[40] Further studies in mice and rats indicated that increasing the activity of a naturally occurring elastase inhibitor, elafin, would have the same beneficial properties[41] without the hepatotoxicity of synthetic inhibitors. In addition to inhibition of elastase, elafin has additional favorable properties: it inhibits the proinflammatory transcription factor NFkB and it is an antimicrobial agent. Elafin also promotes signaling through BMPR-2 by increasing the interaction of BMPR-2 with caveolin.[42] A Phase 1 clinical trial in healthy volunteers has been completed (ClinicalTrials.gov Identifier: NCT03522935) and further preclinical testing will be completed in advance of a Phase 2 clinical trial.

CONCLUSIONS

The exponential increase in PAH research has led to an array of potential new therapies that hold promise as they target biologic mechanisms causing structural changes in the lung circulation. It is anticipated that further advances in genetics will accelerate progress. The discovery of more disease-causing mutations and modifiers as well as treatment-related and PAH-related polymorphisms will help in stratifying patients likely to benefit more from one therapy versus another. Advanced bioinformatic tools are becoming available to better integrate and distil complex epigenetic, metabolic, and proteomic networks, thereby directing us to new therapeutic targets. Advances in cell and structural biology should produce compounds in which the beneficial effects can be better separated from adverse consequences, and in which mitigating a process and restoring balance would be the objective, rather than completely abrogating a biologic pathway. Most important will be the inclusion of multiple biomarkers in clinical trials so that it can be established early on whether the expected disease target was in fact suppressed by the dose and dosing schedule used and, retrospectively, whether there is an explanation for unanticipated detrimental or beneficial off-target effects.

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Limitations and Flaws of Preclinical Pulmonary Hypertension Studies

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Despite advances in our understanding of the disease, significant therapeutic gaps remain for pulmonary arterial hypertension (PAH). Indeed, no cure exists yet for this devastating disease, and very few innovative therapies beyond the traditional pathways of endothelial dysfunction have reached late clinical trial phases in PAH. While there are inherent limitations to the currently available animal models of PAH, the delayed translation of innovative therapies to the clinic may also relate to flawed preclinical research methodologies. The present article discusses the limitations and flaws in the design of preclinical PH trials and discusses opportunities to create preclinical studies with improved predictive value in identifying key mechanisms involved in PAH development and progression and guiding early phase drug development in PAH patients. The implementation of rigorous study design will need support not only from researchers, peer reviewers, and editors, but also from academic institutions, funding agencies, and animal ethics authorities.

INTRODUCTION

The number of scientific publications related to pulmonary arterial hypertension (PAH) increased exponentially over the last decades, leading to significant advances in our understanding of its pathophysiology[1] and management,[2] allowing the delay of clinical worsening,[3] and likely improving survival.[4] However, long-term prognoses of PAH patients can be further improved.[5-7] Significant translational and therapeutic gaps between preclinical research and improved patient outcomes thus persist, as very few innovative therapies have reached late clinical trial phases in PAH.[8-11] This translational gap is not unique to PAH. Since the beginning of the millennium, the number of new drugs approved yearly by health authorities has declined[12] despite marked increases in total research and development expenditures.[13] Clinical drug development is notoriously arduous, with fewer than 5% of *high impact* basic science discoveries[14] and fewer than 10% of development paths in Phase 1[12] being eventually approved by health authorities. Several reasons may explain this phenomenon, including higher regulatory efficacy hurdles and increased complexity and cost of clinical trials.[15] There are also inherent limitations to the currently available in vitro and animal models, which imperfectly mimic the full spectrum of the human disease.[16] Moreover, it has been proposed that the failing might also be related to the study design, implementation, and analysis, ultimately weakening our confidence in preclinical studies to identify promising therapeutic targets.[17] Bias in study design, analytical methods, and reporting practices may indeed compromise scientific validity[18] and data reproducibility,[19] and ultimately jeopardize translation to human studies. Given the limited financial resources, the persistent medical need for improved therapy in PAH, and the restricted study population available for clinical trials, there is a need for reducing the number of false positive signals in preclinical studies and for optimizing the development of innovative therapeutic targets through performance of clinical trials based on more robust experimental data.

Key Words—pulmonary arterial hypertension, pulmonary hypertension, animal models of human disease, methodological rigor, reproducibility, confirmatory study, preclinical study, statistical analysis plans
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RESEARCH BIAS

In research, bias occurs when "a systematic error is introduced into sampling or testing by selecting or encouraging one outcome or answer over others."[20] Bias can cause estimates of association to be either larger or smaller than the true association; in some cases, bias can even cause a perceived association that is directly opposite of the true association. Importantly, bias is relatively independent of both study power and statistical significance in contrast to imprecision, which relates to a random error. Thus, studies may produce precise but biased results because of flaws in study design and execution. Conversely, a study may be free of significant bias but yield an incorrect effect estimate due to low statistical power. While some degree of bias is nearly always present in a study, researchers should make every effort to identify, quantify, and/or eliminate bias through proper study design and data analysis, and to acknowledge its occurrence when unavoidable.

IDENTIFYING AND LIMITING BIAS IN IN-VITRO PAH PRECLINICAL STUDIES

The access to human samples of high quality from PAH patients and appropriate controls represents an invaluable resource for improving our understanding of PAH and validating emerging hypotheses. However, human PAH tissues and cells have been most commonly obtained at the time of lung transplantation from patients with long-standing disease that may no longer be representative of mechanisms accounting for PAH development or progression, introducing a significant selection bias (Figure 1). More importantly, most experimental studies using human samples are performed with small sample sizes due to the scarcity of specimens. Given that PAH is a heterogeneous disease in terms of background genetic defects, concomitant diseases predisposing to PAH, as well as genetic variations influencing response to therapy,[11,21] limited sample size may easily amplify the effects of selection bias and lead to erroneous conclusions (Figure 2A). Therefore, collaborative studies allowing the exploration of promising targets and the real interindividual heterogeneity in a larger number of samples are thus essential (Figure 2B).[22] The creation

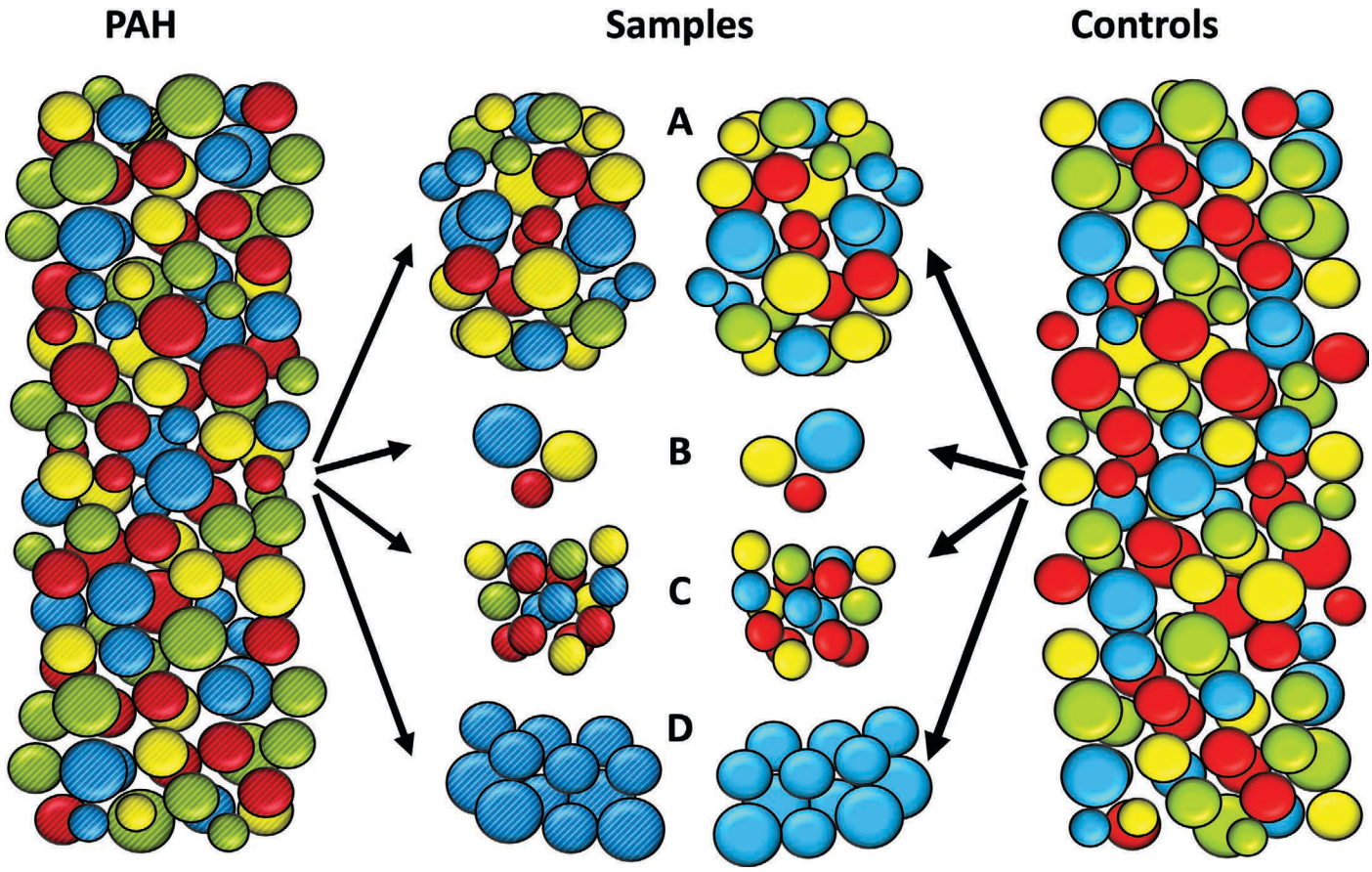

Figure 1: Selection bias in research. In research, selection bias frequently occurs when a systematic error is introduced by sampling methodologies. (**A**) Selecting a sample that is truly representative of both diseased subjects and matched controls is thus mandatory before observed differences are believed to be representative of the disease process, and the observed results should be considered to only apply to subjects with characteristics comparable to the study population. This is most commonly performed using a large representative and random sampling. In pulmonary arterial hypertension (PAH), human tissues and cells have been most commonly obtained at the time of lung transplantation from patients with long-standing disease that may no longer be representative of the mechanisms accounting for PAH development or progression, introducing a significant selection bias. (**B**) Using small sample size or (**C-D**) biased samples (eg, samples from only men or patients with end-stage disease) is unlikely to be representative of mechanisms accounting for PAH development or progression, or to take into account the significant heterogeneity of the disease in humans.

of a structured PAH network (eg, the Pulmonary Hypertension Breakthrough Initiative or the International Consortium for Genetic Studies in PAH) and biobank facilities dedicated to harvesting and preserving explanted lung tissues, facilitating access to human tissue, and ensuring homogeneity in tissue processing, is thus warranted since human tissues are currently underexploited in PAH experimental research.

The choice of the cells and tissues to which PAH samples are compared is also crucially important. Indeed, there are often systematic differences between the groups being compared, known as confounding, so much so that differences in signaling pathways or outcomes may result from these differences rather than actual pathobiological abnormalities. Minimizing these inherent differences is thus essential. Control samples should ideally be matched for age/sex and for their underlying disease. In many cases, PAH researchers have relied on resected lung tissue for cancer. However, special attention is required to obtain tissues sufficiently distal from the tumor that may significantly influence the phenotype and genotype of neighboring cells.[23] In all cases, equivalent tissue specimens should be collected from the same organ areas. This is particularly important within the lungs as distal versus more proximal pulmonary arteries may significantly differ phenotypically. In addition, the same handling and processing has to be used. Taken together, careful selection of control tissues that most likely represent healthy lungs/tissues is crucial.

IDENTIFYING AND LIMITING BIAS IN IN-VIVO PAH PRECLINICAL STUDIES

Despite the importance of scientific results obtained from animal models, most of these studies have been hampered by the fact that these models do not entirely encompass the typical features of human PAH. This may explain why animal models are frequently considered poor predictors of whether an experimental drug can become an effective treatment. Sometimes, though, the real reason is that confirmatory preclinical studies were not rigorously designed. Accordingly, the statistical and methodological rigor should be adapted, and in many ways, confirmatory studies should resemble clinical trials. Indeed, only the most rigorously conducted trials can completely exclude bias as an alternate explanation for the promising results observed following an intervention. Thoughtful subject eligibility criteria, sample size estimation, randomization and treatment allocation concealment, blinding, standardized outcome assessment, proper data handling, and transparent reporting methods have profoundly improved the validity of clinical trial results over the years.[24] Such improvements are also essential in confirmatory preclinical research.

Matching Models to Human Manifestations of PAH

Recruiting a study population representative of future patients to be treated while minimizing confounding effects is the first step of an appropriately designed prospective study. Despite the limitations of current animal models previously discussed,[16,25] a detailed characterization and reporting of animal traits at baseline and appropriate controls using animal characteristics that are representative of the human disease should be promoted for a better standardization of the experimental design, enhanced reproducibility,

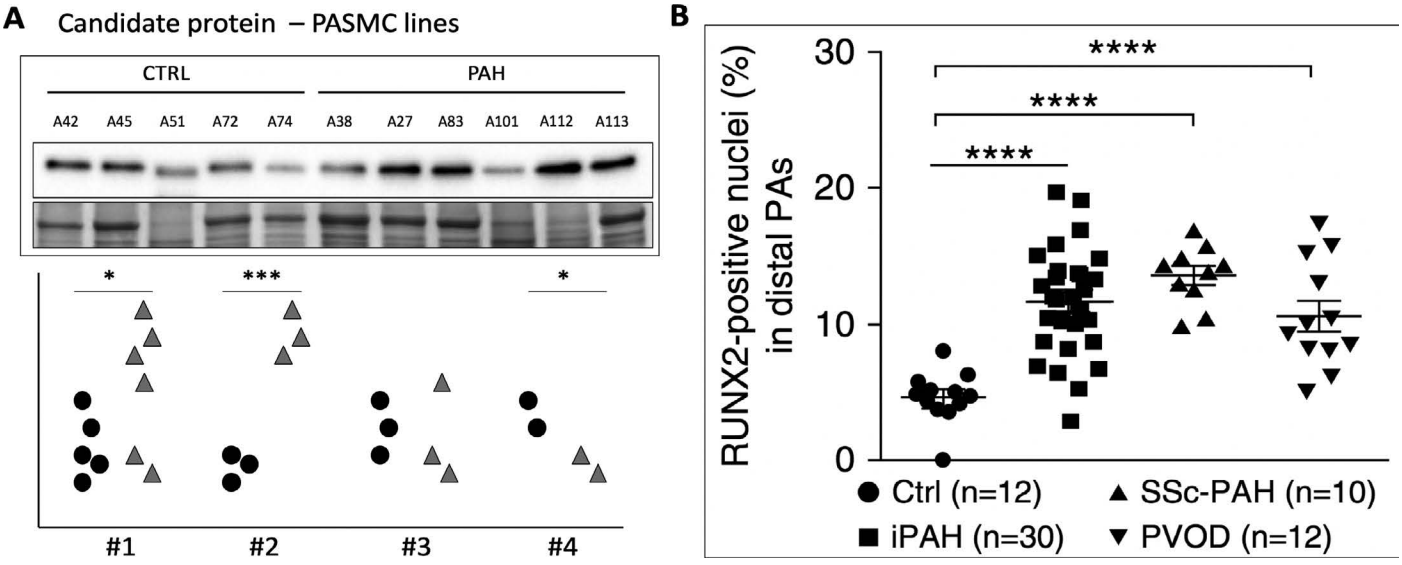

Figure 2: Influence of selection bias and sample size on the presumed validity of pulmonary arterial hypertension (PAH) preclinical results. PAH is a heterogeneous disease in terms of background genetic defects, concomitant diseases predisposing to PAH, and genetic variations influencing response to therapy. (**A**) While even large sample size may be associated with significant selection bias, this may easily be amplified with limited study samples. For example, experiment #1 suggests that the expression of a candidate protein is increased when evaluated on 5 independent cell lines. These results appear to be overestimated when the analysis was restricted to 3 selected cell lines (experiment #2), whereas no difference (experiment #3) or even a downregulation of the candidate protein (experiment #4) were observed when other cell lines were selected. (**B**) This is exemplified in a recent collaborative work to assess the role of RUNX2 in a large number of PAH samples.[22] In that study, significant overlap in the proportion of RUNX2-positive cells was observed between PAH and control tissues obtained at the time of lung transplantation. Although such analysis may not be representative of the early disease processes, a smaller sample size would have increased the risk of overestimating or underestimating differences between groups.

and greater predictive ability. Currently, variations in disease induction and the potential for persistent and unrecognized confounders, including considerable inconsistencies in animals' ages and weights, how pulmonary hypertension (PH) is induced, and when the intervention is initiated and terminated,[26] represent important sources of bias in PH preclinical research. Care must thus be taken to prespecify eligibility criteria before animals are enrolled. In confirmatory preclinical studies, it is also reasonable to randomize animals to novel therapies when irreversible PH is expectedly fully established and following prior confirmation (eg, by echocardiography). In addition, the rationale for choosing models should be stated,[27,28] and performing studies using more than one model and across different animal strains is encouraged. Ultimately, large animal models may share some common features of the human disease and are often the last step before translating novel drug candidates to clinical trials.[29] While the need to include women is now a well-established requirement in clinical trials,[30] analogous standards have not been equally enforced in preclinical stages of research. While the vast majority of preclinical PH studies still use male rodents only,[26,31] inferring experimental findings to both sexes when a single sex is studied could disadvantage women by biasing our understanding of disease processes toward male-predominant patterns. This is especially problematic in PAH, where there is a significant female predominance in humans.[32] The landscape of clinical trials in PAH also dramatically changed over the last decade, and future compounds will almost necessarily be tested on top of currently available therapies in clinical trials leading to drug approval.[2,33] Although new targets can be alternatives to the currently approved therapies in humans, the demonstration of additive or synergic effects of novel therapeutic targets nowadays appears desirable for confirmatory preclinical studies.

Randomization and Allocation Concealment

The starting point for an unbiased interventional study is the use of a mechanism that ensures that the same sorts of *participants* receive each intervention. Even an apparently homogeneous group of animals may have inherent differences when the intervention is introduced. Thus, processes need to be considered to allow proper balance between groups and, as for humans, random animal allocation generally minimizes bias and balances characteristics that may influence response to treatment if properly done in a large enough sample. Techniques used to implement the allocation sequence (ie, allocation concealment) are also essential to avoid selection bias being introduced by selecting animals based on the upcoming intervention assignment. There is indeed empirical evidence from preclinical research[34,35] that either inadequate generation or concealment of allocation sequence yield to exaggerated estimates of intervention effects. Therefore, researchers should ideally report measures of successful randomization and allocation concealment.

OTHER POTENTIAL BIAS IN PAH PRECLINICAL STUDIES

Blinding of Outcome Assessment

Blinding refers to the process by which the study personnel are kept unaware of intervention allocations. Lack of blinding in clinical trials is associated with exaggerated estimates of intervention effects,[36] especially when the outcome of interest is subjective.[37] Importantly, many apparently objective outcome measures in preclinical PAH studies remain subject to interpretation. Unconscious bias can thus creep into evaluation of unblinded experiments even when performed by scientists of high integrity. Although blinding the investigator administering the treatment/intervention may not be possible in all instances, blinded assessment of imaging, hemodynamics, and histological outcomes is almost universally possible through independent team members performing outcome ascertainment.

Study Readouts and Interstudy Standardization

Even with rigorous attention to study design, studies may not have translational validity if the endpoint specified is not valid or is not measured using robust techniques. Importantly, outcome measures should match the clinical realm using relevant measures (eg, comprehensive hemodynamics in in-vivo studies). Secondary readouts are generally used to provide supportive information (eg, to ensure hemodynamic endpoints are correlated with histological, anatomic, and biochemical findings postmortem) or exploratory, hypothesis-generating information. Obviously, the exploratory nature of some experiments makes sample size calculation impossible or meaningless. Conversely, the importance of prespecified sample size calculation, referred to as a power calculation, in confirmatory experiments cannot be overemphasized, although it is rarely performed in preclinical PH studies.[26,31] Using human samples or exposing lab animals to research is only justifiable if there is a realistic chance that the study will yield useful information. Importantly, inappropriate samples will result in an inconclusive study, whereas an unnecessary large sample size will accrue excessive cost. Many researchers are thus tempted to perform interim analyses to subsequently increase the sample size as necessary. However, interim analyses enhance the risk of false positive results due to multiple analyses. Therefore, the primary endpoint of preclinical confirmatory PH studies must be decided before the study begins, as well as the effect size of the intervention for which the study is powered, and should be provided in the *methods* section of confirmatory experiments. Similarly, empirical work has confirmed marked heterogeneity in the methodologies used to assess study outcomes in preclinical PH studies,[26] including pulmonary hemodynamics, markers of right ventricular function, and pulmonary remodeling.[26] The majority of in-vivo studies also fail to appropriately monitor for toxicity. Obviously, these elements cannot be unilaterally dictated but require a consensus process to take place, with experts in the field agreeing on best practice, as has been previously developed for preclinical in-vivo evaluation of pharmacological active drugs in other fields.[38-40]

Multiplicity, Interim Analyses, and P *Value Adjustments*

Because multiple readouts are necessary to fully evaluate pathophysiological

pathways and the effects of interventions, multiple endpoints are frequently measured. However, conducting multiple tests of significance progressively increases the probability that a null hypothesis is rejected when the null hypothesis is actually true (ie, false positive result). Consideration must be given to controlling the risk of false positive conclusions, and adjustment for multiplicity will typically be necessary, especially for confirmatory studies.[41-43] Similarly, interim analyses frequently used to incorporate what is learned during the course of a study increase the risk of falsely rejecting the null hypothesis. Researchers should thus avoid unplanned interim analyses, and preliminary results should be presented without formal statistical analyses unless nominal *P* values have been adjusted accordingly. Collaboration with a statistician at the design stage and throughout analyses is thus crucial, and the selected procedure must be prespecified in the statistical analysis plan before undertaking any analyses of the data.

Handling of Missing Data

Attrition and exclusions frequently occur in preclinical PH studies when animals die or are withdrawn from the experiments or assessment does not provide relevant data. The risk of bias from incomplete outcome data depends on several factors, including the amount and distribution of missing data across intervention arms and the reasons for missing outcome data. Researchers should consider using a flow diagram showing the number of animals in intervention and control groups at each experimental step from randomization to outcome assessment. A timeline of experimentation is also desirable to inform whether all animals within each experimental group were analyzed together. For confirmatory studies, an intention-to-treat analysis may be considered as potentially the least biased way to estimate intervention effects in randomized trials.[44] However, true intention-to-treat analyses generally require imputation, which can also lead to serious biases unless conservative methods are used. Thus, where imputation is used, both the *per protocol* and the *intention-to-treat* analyses should be presented, and the methods and assumptions for imputing data should be defined a priori and appropriately described.

Interpretation of the Results

The high pressure to find low *P* values, combined with a common misunderstanding of how to correctly interpret *P* values, frequently distorts the interpretation of *significant* results.[45] A low *P* value is considered strong evidence against the null hypothesis. However, a *P* value of .05 is frequently incorrectly interpreted as meaning that there is 95% chance that the observed difference is true, rather than indicating a 5% probability that the difference is observed even if the null hypothesis is true. Previous studies estimated that a *P* value of .05 corresponds to a false positive rate of *at least 23%* (and typically close to 50%).[46] Thus, a single statistically significant hypothesis test often provides insufficient evidence to confidently discard the null hypothesis, and study replication, especially by independent investigators, enhances the confidence that study results are true findings. Therefore, investing more time in replicating results (those of others as well as our own) and synthesizing data through systematic reviews and meta-analyses should be incentivized.[47,48]

Reporting and Publication Bias

Reporting biases arise when the dissemination of research findings is influenced by the nature and direction of results. While publication bias (occurring when entire studies are not published, are published in obscure journals, are rarely cited, or are inappropriately indexed in databases) is the most obvious form of reporting bias, *within-study publication bias* may be one of the most substantial biases affecting results from individual studies,[49] analyses with statistically significant findings, or extensive magnitude of effects being more likely to be reported compared to *uninteresting* or *unwelcome* findings. Reporting bias almost inevitably leads to major overstatements of efficacy,[50] including in preclinical PH research.[26] Intriguingly, selective submission by the authors rather than selective acceptance by the reviewers may predominantly contribute to publication and reporting bias.[51,52] Conversely, some journals indirectly contribute to this phenomenon by relegating *less interesting* findings to the supplement section. Publication and selective reporting biases also prevent others from learning about negative study results (which, by the way, should be allowed by editors to be published even in big impact-factor journals), with implications for animal ethics and research funding. To minimize publication and reporting bias, study preregistration was developed for clinical trials, limiting researcher ability to modify planned experimental design and analysis afterwards. As a result, the International Committee of Medical Journal Editors now considers only those clinical trials for publication that have been registered before the start of patient recruitment.[53] In preclinical studies, preregistration in a public repository at the study inception is a debated issue. Indeed, while a finding is more convincing when it was predicted, breakthrough findings have been made through exploration with limited a priori hypotheses.

ADAPTING STATISTICAL AND METHODOLOGICAL RIGOR TO THE PROGRAMMATIC PURPOSE OF RESEARCH

While investigators seek to provide a better understanding of the pathophysiological processes and identify key cellular and molecular signaling pathways/targets involved in disease development in exploratory research, detailed and reproducible information on efficacy, dosing, and toxicity of potential drug candidates are required in confirmatory investigation to decide whether the drug could be tested in clinical trials. The statistical and methodological rigor should thus be adapted according to the nature of the study (Figure 3). Nonetheless, even at an exploratory stage, significant attention should be paid to identify, avoid, and acknowledge potential bias.

A CALL FOR CHANGES IN PRECLINICAL PH STUDIES

Scientific irreproducibility is a growing concern among academics and in the general population.[55] Biases and poorly

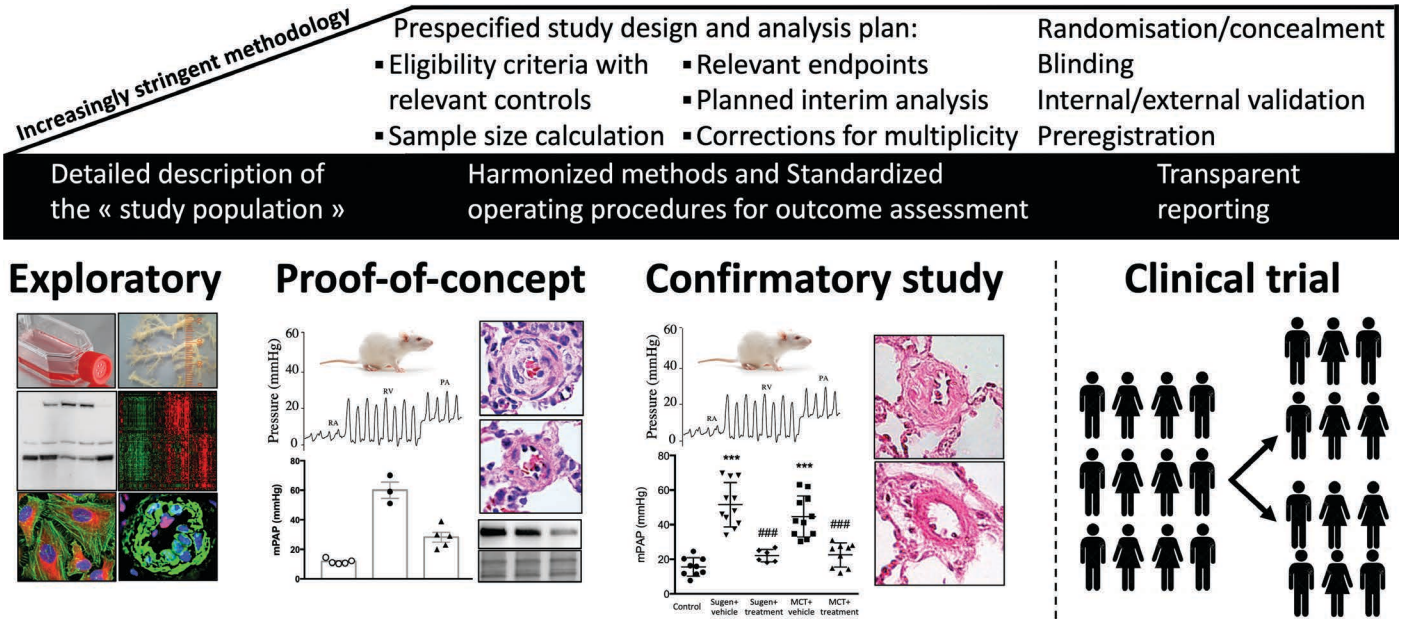

Figure 3: Adaptation of statistical and methodological rigor according to the nature of the study. For confirmatory studies, the most rigorous application of randomized controlled trial–like approaches should be applied, including prespecified study design and a statistical analysis plan describing eligibility criteria, sample size calculation, relevant study readouts, interim analysis (if any), and corrections for multiple analyses, as well as randomization and concealment procedures, validation of the results, and potentially study preregistration. However, this type of approach may be expensive and cumbersome, and applying randomized controlled trial–like standards to studies focusing on discovery and elucidating fundamental biological processes may not add the same level of value. Thus, methodological rigor must be appropriately calibrated to the goals of the study. In all cases, however, the choice of an appropriate animal model that is representative of the human disease, the development of standardized operating procedures, blinded outcome assessment, and transparent reporting are critical.[54]

designed preclinical studies likely contribute to experimental irreproducibility, wasted resources, and erroneous conclusions.[56] In response to these issues, the National Institutes of Health (NIH) proposed a set of guidelines and funding policies as minimum reporting requirements to promote rigor, reproducibility, and transparency of preclinical research that have been endorsed by prominent academic societies and scientific journals with editorial commitment to complying.[57-59] These include guidelines and checklists to improve methodology and reporting.[57,58] Multicenter preclinical studies,[47,60] systematic reviews, and meta-analyses are also preconized.[48,61] Practical solutions to improve preclinical research quality and research translation have also been specifically proposed in PH preclinical studies.[16,54] Implementing such requirements will involve a paradigm shift for scientists, their institutions, journals, and funding agencies.

CONCLUSION

In preclinical research, methodological sources of potential bias and imprecision are prevalent and frequently overlooked by researchers, potentially contributing to the significant discordance between preclinical and clinical results. Although not unique to PAH, concerted efforts to address this problem are needed for more effective translation of preclinical research findings into sustainable improvements in patient outcomes, including rigorous study designs, methodological standardization, appropriate data interpretation, and statistical analysis plans, as well as transparent reporting of preclinical studies.

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Drug Development and Negative Clinical Trial Results

This May, Guest Editors Marc Humbert, MD, PhD, Director of the French Pulmonary Hypertension Reference Center, and Professor of Medicine at Paris-Saclay University, Le Kremlin-Bicêtre, France, and Mark Nicolls, MD, Professor of Medicine, Pulmonary, Allergy, and Critical Care Medicine at Stanford University School of Medicine in Stanford, California, held a discussion with Norman Stockbridge, MD, PhD, Director of the Division of Cardiology and Nephrology in the Office of Cardiology, Hematology, Endocrinology, and Nephrology at the Center for Drug Evaluation and Research, US Food and Drug Administration in Silver Spring, Maryland, and Roham Zamanian, MD, Associate Professor of Medicine and Director, Adult Pulmonary Hypertension Program at Stanford University in Stanford, California, on the topic of clinical trials, drug development, and publishing negative trial results.

Dr Nicolls: Thank you all for being here today. Let me open the discussion. There have been several negative clinical trials that have been performed in the pulmonary arterial hypertension (PAH) space. I would like Roham to comment on how important he thinks it is to publish the results of negative trials. What can we learn from negative trials, and do you have any ideas about how we might develop leverage for getting the data out?

Dr Zamanian: I think we should start by recognizing that we are discussing a rare disease field with a number of stakeholders: patients, patient advocates, clinicians, scientists, academicians, industry, regulators, clinical trialists, and even policy makers. Obviously, the concern is that there is a general bias against "negative" clinical trials, and those biases may be different across stakeholders and also impact them differently. I think maybe this issue is much more pronounced in a rare disease field where there is a limited number of subjects to study and tremendous competition for funding studies which are often costly. The bias not to publish negative trials is a tremendous loss for the community, especially with pulmonary hypertension (PH), where there are so few subjects and they're very expensive to study in an efficient and appropriate manner.

The other thing I want to say, and probably this is not groundbreaking, is that a study can be deemed a negative trial when it either has constraints in its design or its implementation, or it does not appear to show what the investigators had hoped and had hypothesized that it would. Therefore, it's brushed under the table, especially if reporting of the trial negatively impacts the financial wellbeing of a sponsor or the reputation of investigators.

Dr Nicolls: Can you give some examples of things that we can learn from negative trials beyond the fact that the drug in question didn't reach its primary endpoint?

Dr Zamanian: I think we could learn either that a mechanism is not relevant to the disease, or that the disease doesn't respond in the way that we thought it would. More importantly, we can learn a lot about trial design, from selection of endpoints, use of specific enrichment strategies, to trial conduct and challenges with subpopulation recruitment. All trials, positive or negative, can be tremendously informative. To be able to perform a "postmortem" as to why a study is negative is crucial, and it could move the field forward, so we need to overcome the misconception that a negative trial is a "failed" experience.

Dr Nicolls: Dr Stockbridge, maybe I can ask you to jump in on this point. I don't know if you have an opinion on publishing negative trials from your vantage point; it's not really what you're most involved with, but do you have any comments on anything Roham said?

Dr Stockbridge: We're talking here about nontrivial trials. I don't think that anybody cares quite so much about the first in-man and Phase 2 exploratory studies, but trials that actually had a reasonable shot at establishing effectiveness. It's important to note that a healthy clinical trial therapeutic area development ecosystem has to have trials that fail. If you win on every trial, if that's all that ever happened, then you're not doing enough trials. It's an important aspect that things do fail, and people need to understand something about what happens there.

One aspect of this is whether the investigators going into a trial have negotiated the freedom to publish regardless of the outcome. I urge clinical trialists to make sure that they retain the right to publish. You don't want the company constraining that; even if they have some input on it, they should not constrain it.

The other aspect of this is that I think patients who invest their time and energy and risk in participating in these trials don't generally think they're doing it to support some company. They generally think they're doing it to support medical knowledge in some area. If we don't publish those studies and make use of what they've invested, I don't think we're doing what we're supposed to with respect to them.

Dr Nicolls: That's a great point. Building on that question, can you use your imagination to think of any kind of leverage that might be exerted in the future to compel companies to release the information? Roham and I are actually involved in a situation like this, so we would love to publish the results of our negative trial and dig into the data. I think Roham was interested in

the subject even prior to that experience, so it's not just because of that. What kind of leverage or language would be helpful? I think sometimes the problem might be that the company is not releasing the information because they believe that it will in some way be bad for their stockholders to have a negative trial published.

Dr Stockbridge: I think it's too late when the trial's over. After you sign the contract, it's too late. The place to be working on this kind of problem is setting up groups that have a strong enough presence in the field that companies are likely to come to them to get a trial done. Then you're in the driver's seat. You have some ability, at that point, to negotiate how the contract is written, to ensure that you've got access to the data, and some privileges with regard to publishing no matter how it comes out. If you wait until it's over, you're going to have a hard time.

Dr Nicolls: Because not everyone is in the luxurious position of having that kind of international reputation, and sometimes these companies are one-off companies that may not come back with another PH drug, I think something that anybody could do, any investigator-initiated proposal, would be to start at the contracting process before the trial. I think that could be a good takeaway point from this discussion.

Dr Zamanian: At the World Symposium on PH in 2018 in Nice, France, Dr Stockbridge and I and others, including Lewis Rubin, were on the same task force, and from that section came the recommendation that you develop a process for reporting negative clinical trials, you make it a contractual obligation up front and utilize the leverage of international experts who carry a lot of weight in that process. The second part to that is that you may also need to develop an independent and self-funded core, whether that resides as part of the data safety monitoring board or the steering/adjudication committee or an analytic core committee that would carry out and report on analyses, so that you're not depending on the industry for providing statistical support. Tie that in with some sort of a mandatory publication timeline which balances the need to report on a study with the industry need to absorb the findings—I would suggest something like a publication embargo period of maybe 6 months so that the impact of the data on the company may be minimized. Really, the stockholders will know by 6 months after the study whether the study was negative or positive.

Dr Nicolls: That's great. I think maybe we should move onto another subject. I'll invite Marc Humbert to introduce any topics he'd like to discuss.

Dr Humbert: I wanted to focus a little bit on Phase 2 studies in PAH because we usually have different endpoints in Phase 2 and Phase 3 clinical trials, and the negative trials give us different messages. I was thinking that the relatively small Phase 2 studies, in which primary endpoints are usually pulmonary vascular resistance (PVR) measurements obtained by invasive right-heart catheterization, should always be presented in great detail with careful presentation of individual data, not only because we want to have a complete analysis of the active compound results, but also because of the importance of the results observed in the placebo arm, or the control group. I wonder whether either Dr Zamanian or Dr Stockbridge would like to comment on the possibility of having a single control group for several active drugs tested in order to expose fewer patients to a placebo, especially when data obtained by right-heart catheterization are needed.

Dr Zamanian: I don't know if I have an answer, and I would love to hear Dr Stockbridge's opinion on this. Several of us have discussed the idea of using a master protocol in early phase development as a platform for what you've just said, Marc. The idea is to test multiple drugs more efficiently and allow for a single placebo arm that would be used for a single control arm and others which are the intervention/therapeutic arms. This would allow a more efficient drug development program and allow for some degree of adaptation, utilizing biomarkers or maybe some phenotype information.

The difficulty with the idea of the master protocol is not, I think, a problem for academicians. I think when it comes to the biopharma companies, they may view it as competing with each other. I would love to hear Dr Stockbridge's thoughts about utilizing that in the setting of a master protocol.

Dr Stockbridge: We're certainly on record as having advocated for people to do that as a way to get efficient Phase 2 studies done. I think the reason why it doesn't happen is because nobody knows how, or nobody knows what to do to organize such a study. I think the problem has been getting companies that are not anxious to share and not anxious to even be perceived as helping to move the field, move their colleagues along, move their competitors along. It's hard to bring them to the table. They need to be more or less at the same stage because the first company doesn't have a lot of incentive to help drag the rest of the community along. This does need to be organized, I think, at the academic consortia level or some international funding agency or something, and then you're going to have to sell it to the companies.

Dr Humbert: It's really interesting. I think that patients should be really strong advocates for that in close partnership with other stakeholders. Maybe the academicians, as Roham said, should try to develop such master protocols and persuade the National Institutes of Health (NIH) or some European funding agencies to support this. Of course, it's difficult to organize, but I think it would be very good to do it as soon as possible, or least try to, because a big disappointment for me is to see multiple, very small Phase 2 studies with invasive measures first presented as negative on press release, but with no data whatsoever presented to the community. I feel that it's a big waste, and I think it's not really ethical to keep these data hidden somewhere.

Dr Nicolls: I agree. We're about halfway through our time here, so Marc, I have three different ideas for the next discus-

sion. One is, I'll always remember being at a meeting with Norman Stockbridge and talking about the utility of using the 6-minute walk distance.

Another subject is the challenges of add-on (ie, adjuvant) therapy in the 21st century, when we add in a new potentially disease-modifying therapy on top of maximal vasodilation. It can be very, very challenging to get a readout; even though it might be an important therapy, the ability to detect change in a rare disease is challenging.

The third topic is, is there a value in developing endotypes for a rare disease, like looking for responder subsets in an already rare disease?

What do you think would be the most interesting one for us to discuss?

Dr Humbert: I think the patients would like to hear from experts about the typical classical endpoint like 6-minute walk distance, and then we can touch on the other subjects. I would like to discuss a little more the 6-minute walk distance as a Phase 3 endpoint, either on its own or as part of a composite endpoint.

Dr Nicolls: Can I ask it in the form of a controversial question? Basically, some believe that the 6-minute walk distance is an overly crude measure of determining whether a drug is useful. The Food and Drug Administration (FDA) has historically been very much in favor of using this benchmark, and it's time-honored and useful. It's certainly usable. Why don't I ask Roham to go first?

Roham, what is your view of the 33-m idea, and what do you think about where we are as a field with regard to endpoints?

Dr Zamanian: I'm not sure if I specifically should comment on the 6-minute walk, but generally, I think one of the challenges we currently have with endpoints is that it can be difficult to show a treatment effect. Let's assume that's about 6-minute walk distance. We now have the largest number of patients in therapies that are on at least dual therapy from a vasodilator perspective. Most of the current trials enroll new patients on these therapies with New York Heart Class 2 or early Class 3 symptoms.

I think, from a 6-minute walk distance perspective, it may be valid to say that we're challenged, because of existing success with vasodilator therapies, to show a treatment effect going forward. On the other end of it, I guess, if you accept that argument and you move toward clinical worsening, whether just purely mortality or a composite endpoint, those studies become extremely difficult and expensive to perform in our rare disease population. I think that challenge that I see is not entirely just the 6-minute walk.

I would love to hear Dr Stockbridge's opinion on what the future would be. Mark, I think you and I have been involved with a number of studies, both academic and some early-phase industry, where I think that the fact that we have these patients, even those on intravenous therapies, who are maximally vasodilated, makes it increasingly difficult to pick up a signal of a 6-minute walk distance change with the number of patients we keep enrolling into these Phase 2 studies, which is about 100 to 150 patients.

Dr Nicolls: Before I turn it over to Dr Stockbridge, do you want to just quickly comment on this 33-m cutoff? Because you'll recall that there are some trials that have moved forward to Phase 3 with a 10-m walk difference, which turned out to be—I think Phase 3 was terminated. Do you think that's reasonable, or do you think that's unreasonable?

Dr Zamanian: I think some of those decisions are based around the appetite for risk and what investigators or decision makers of those studies will accept as a potential "signal" without meeting this sort of a bright-line rule of a *P* value of .05. I believe Steve Mathai's publication is a study that shows the minimum important difference for a 6-minute walk distance in a connective tissue disease population is around 33 m.

I know Marc and others have published also on what's appropriate and meaningful change in 6-minute walk distance. I think those decisions might be both a scientific and a business interpretation of the data in early phase studies.

Dr Nicolls: Great. Maybe I'll turn it over to you, Norm. Any thoughts?

Dr Stockbridge: First of all, I need to respond to this notion that we've promoted the 6-minute walk. We have not. Somewhere along the line, somebody came in and asked if it was acceptable, and we said, "I guess so." Because it was successful, other people followed suit. At no point did we allege that this was the best, or the only, or anything more than an acceptable way to demonstrate the benefit.

There is another thing that's kind of interesting here, and I don't think there's anything written about it from a regulator—some of this obviously took place while someone other than me was a division director.

If you were to come in today with a more common disease and say, "I want to do a 6-minute walk," we would tell you that it's fine, but you need to show us an effect that a patient has some opportunity to perceive. We never did that with PAH. We didn't do that initially, with the first few drugs, and we didn't start incorporating it until we were beginning to get asked about the effects on the order of 10 m. I think the 6-minute walk effects that have been seen with vasodilator therapy have been tiny, have been miniscule.

What I am sure of is that, because we didn't ask for big effects, we got a fair amount of drug development done here, an opportunity to look for things that were perhaps more clinically meaningful—disease progression kinds of endpoints, hospitalization endpoints—and I think we created some interest that will now drive the field more toward disease-modifying kinds of therapy. We probably wouldn't be where we are if we had taken the classic approach of insisting that an effect be demonstrated that was actually clinically irrelevant.

Dr Nicolls: That's a really fascinating discussion. The way that I was understanding it was that the FDA looked at the 6-minute walk distance as the "gold standard" for large trials. That was my bias. That's what I've always thought.

Dr Stockbridge: Never happened.

Dr Nicolls: Interesting. I'm just curious whether or not Roham or Marc was laboring under that misunderstanding as well, if you thought that the FDA thought that the 6-minute walk distance was the "gold standard" for the field.

Dr Humbert: Yes, I think we have understood that the message that the 6-minute walk distance was quite a pragmatic way to favor drug development in a feasible fashion in rare conditions like PH, and we recognize that it has been extremely helpful. That's something we appreciate very much.

The problem we face right now is, what can we offer as an alternative that will support drug development and innovation efficiently? When we develop clinical trials, we often discuss composite endpoints such as "time to clinical worsening" or "time to treatment failure," etc. To be frank, it has a negative aspect in the wording, while walk distance is expressed as improvements, which is more positive. I think that we all wish to develop a more positive endpoint of "clinical improvement," but it is quite challenging, and we often end up with a combination of parameters which usually includes walk distance improvements and biomarkers which have no direct consequences for the patient. I must say that I think a lot about endpoints, and I try to find the best way to develop a drug efficiently for my patients. I must confess that, in the last 20 years, the walk distance has treated us quite well and that other endpoints are more complex to use.

I think the walk distance was a good endpoint for early drug development in PAH and chronic thromboembolic PH (CTEPH). For future drug development, we will have to consider using modern technology, maybe actigraphy exercise recording, etc. I know that the agencies are quite open to these kinds of endpoints. We are very far from Phase 3 clinical trials with a positive endpoint on actigraphy in PH, but I hope it will come one day.

Something I wanted to discuss, because Roham said it twice, is the concept of "maximum vasodilator therapy." I would rather say "combination therapy" because I am not sure it should be labeled as "maximum." I would rather say that it's the current standard of care. Right now, we have lots of patients on double or triple combination therapy who still present with very high pulmonary artery pressure, very high PVR, exercise limitation, and a lot of room for improvement in terms of hemodynamics and other clinical endpoints. Rather than saying that they are on "maximum vasodilator therapy," I prefer to state that they receive "double" or "triple combination therapy." Indeed, the word "maximum" corresponds to our current knowledge, but hopefully we will have more to offer sooner or later. Recently, we have seen some quite interesting results suggesting that you may be able to decrease pulmonary artery pressure on top of what we currently consider as "maximum" vasodilator therapy, corresponding to "double" or "triple" combination of vasodilators. Maybe we should think outside the box a little bit and consider that we will be able to add a treatment targeting other mechanisms on top of the current standard of care, leading to meaningful decreases in pulmonary pressure and resistance.

We have been very pessimistic in recent years, certainly too pessimistic. I remember at the 2018 6th World Symposium on PH in Nice, there was a trend to say that all the Phase 2 studies had failed and that it will be difficult to develop something meaningful rapidly. Indeed, it's very difficult to develop new agents in the field, but when you try to understand the true mechanisms at play in PAH, maybe novel pathways which are critical in at least a subset of patients, there is certainly room for significant improvement in our patients because many are very limited in terms of exercise capacity and still have hemodynamic compromise. I think we have to be positive thinkers and try hard to develop drugs which should be efficacious on top of what we have.

Dr Zamanian: I quite agree with what you're saying, Marc. I guess my intent wasn't to say that those are maximized; I think my intent was to reflect that, maybe more than ever, there are now patients who are on dual or triple therapy. As Mark and I are dealing with in an NIH study, there are some clinical trial subjects who now are on dual or triple therapy and have lower PVR than anticipated, meaning that the power calculations that were used to estimate the impact of the therapy on PVR as an endpoint are inaccurate. I completely agree about this notion that we need to keep going because there are patients who have very, very severe disease despite current therapy.

Before we leave the endpoint discussion, can I just ask—there are a number of colleagues who have been working on patient-related outcomes (PROs) and health-related quality of life tools, and we're very aware of a wonderful meeting sponsored by the FDA. In terms of hearing the voices of the patients and using PROs as primary endpoints of clinical trials, I wonder if both Dr Stockbridge and Dr Humbert could speak to that and how we would envision PROs as maybe even primary endpoints of Phase 2 studies.

Dr Stockbridge: There's no problem from my perspective in having a PRO as the basis for approval. If patients say they feel better on some instrument, that's got to be at least as good as 10 m on a 6-minute walk, but it has the same potential problem that, if you've enrolled 800 or even 100 people in a study and show a small effect that appears to be about the same in everybody, that effect will be perceptible to no one. You've got to deal with that if we're going to move into therapies that have more clinical impact than the ones we have now. You ought to want them to show effects that individual patients are apt to perceive. This isn't an issue with hospitalization. I don't care if it's 1/10 of a percent, statistically significant 1/10 of a percent change in hospitalization, but PROs ought to be perceptible to patients.

Dr Humbert: These are very interesting comments. I am very much in favor of PROs, but what Norman just said is very important. The instruments should be of good quality; the effect should be sizeable and meaningful. At the end of the day, I feel that the instruments we use right now with the drugs we currently develop show marginal effects.

This might not be the case for all the pulmonary hypertensive diseases. For example, CTEPH patients treated successfully with surgery or angioplasty will show large improvements in terms of PROs. That's another field, but I think it demonstrates that PROs could be a primary endpoint to demonstrate efficacy of novel agents, but the size of the effect has to be as large in order to convince the community, which is not always the case with drugs we have developed recently; but I remain optimistic that it will come one day.

Dr Nicolls: That's great. Maybe we have time for one or two more subjects. I see that Dr Stockbridge represents cardiology, hematology, endocrinology, and nephrology. Thinking just on the first one, cardiology, there are conditions where you need to layer on drugs, established standard-of-care drugs, but in diseases that may have a log order more patients than we have currently in PAH. This brings us to the challenge of adding adjuvants to the standard of care, potentially disease-modifying adjuvants. There are many different ways that you could take this question, Dr Stockbridge. I'm going to let you take it wherever you would like to.

There is maybe a useful oversimplification in PH—you can reasonably disagree with this statement; I'm not intending it to be dogmatic. Right now, when we give vasodilators, we are treating potentially a symptom and not a pathogenic driver of disease, yet our way forward in the field of actually treating disease-causing factors, such as metabolic changes or inflammatory changes, is extremely difficult because our readout is primarily based on what we think are PVR and things that are already being addressed by the symptom-driven therapy. With the hat that you wear, do you have any ideas for us about how we proceed with adjuvant therapies?

Dr Stockbridge: I'll just mention that in the area of cardiovascular disease and heart failure, the advice we've routinely given is that, if you want to study a new drug, it needs to be on the background of standard of care that includes the other things you know are useful. That's a little bit complicated in the PAH setting where there are a number of things you could be doing that are—I mean, it's more like hypertension, right? You have drugs of different classes, and there may be some questions about which ones reasonably add to one another, but there's also not the expectation that all of the classes get tested. Even there, I think we're going to be forced out of the mode of insisting that new therapy be tested on top of other things. It becomes just infeasible. Even though mortality rates and morbidity rates may be very high still, it's just taking bigger and longer studies as the incremental effects of 10% here and 15% there whittle down on the observed event rates and the trial. I think we've got to be open to the idea of studying these things in a setting where people agree that it's okay not to be on some things you know work as well as plowing some new ground.

Dr Nicolls: That is a really interesting statement. I like it. I don't think it would play well with a lot of people in the PAH clinical trials community, but from my perspective, it sounds interesting. Marc, can you comment?

Dr Humbert: I like the thinking behind the statement, and I have very little to add, but it remains challenging.

Dr Nicolls: Do you think it would be hard to adopt in our field?

Dr Humbert: Could be difficult, yes, because guidelines clearly advocate for combination vasodilator therapy in most PAH patients, and it may be challenging to delay use of combination drugs in symptomatic patients.

Dr Nicolls: Dr Stockbridge, do you see any examples of that happening in cardiology?

Dr Stockbridge: I think people are now just so stressed over the number of complementary therapies that need to be accommodated in this that they're beginning now to talk about nonoptimized background seriously, but there's no precedent for it, and we've set huge, decades' worth of precedents that say, "We expect background therapy to be optimized."

PAH may be, hopefully, on a different pathway. Although the effects seen with other drugs in this field have been small, the therapeutic area is ready for, and people are trying hard to find, the therapy that's actually going to be disease modifying.

You couldn't find a pulmonary-specific vasodilator. There wasn't one. You had to deal with systemic vasodilators. If you can manage the toxicity associated with antiproliferative therapy, you can expect huge effects. Somebody who's got a product in PAH with disease-modifying characteristics is going to be the background for everybody from that point on.

Dr Zamanian: Can I ask you quickly—Marc, you mentioned that this idea might not have traction in our field. Could you maybe outline what some of the challenges would be in implementing this approach in PH? If you were one of the opponents, what would you identify as these challenges that make it impossible?

Dr Humbert: Could you clarify—what was the idea that would be difficult to adopt?

Dr Nicolls: What we're saying is, if we thought we had—let's not use the word adjuvant. Let's say a new disease-modifying drug that might have a long-lasting, positive vasodilator remodeling benefit beyond that of a standard, if there is such a thing as a standard vasodilator. Let's just call it a generic vasodilator. The worldview of the field is that it's widely accepted that whatever we do, we have to add it on top of standard of care. It's written into our subconscious by now. Dr Stockbridge gave a very good, I think excellent, point that maybe it doesn't have to be that way, yet I think that, if we were to introduce that idea, it would meet a lot of resistance. I think Roham was asking, do you agree, or why do you think there would be resistance? Where would it come from? What would it look like?

Dr Humbert: It would be challenging today to ask somebody to stop "gold standard" vasodilator therapy to be randomized to a new agent which might indeed be very efficacious. This approach will need to be very careful. However, I am quite positive that we are developing new drugs which are really fascinating because they deal with new relevant pathways. If some new drugs are working on top of double or triple combination therapy, my strong belief is that it would be interesting to approach future development in two ways. One way would be, if this drug is truly disease-modifying, to consider stopping this new drug and see what's going on in the long run. The other way would be to consider stopping/titrating down the vasodilators that are known to be efficacious, in order to see whether they can be replaced by a new "gold standard."

Right now, in some forms of severe asthma we have developed new quite efficacious drugs, and recent clinical trials have tested either stopping these new drugs and evaluating possible disease-modifying effects or decreasing the standard of care treatments while being treated with these new agents on their own.

Dr Nicolls: It looks like we're back up against our time here, so I don't think we'll have time to get to that last question, but I think this has been a great discussion. Thank you all for participating.

Patient Perspective: Going Through a Clinical Trial

Lena Bolivar
Salinas, CA

When I was diagnosed with idiopathic pulmonary arterial hypertension in late 2011, I was devastated, but I knew I had to be strong for my family. At the time, I had been juggling my life with being a wife and mother to two young children and working full time. I had difficulty with my breathing for years, and even after describing my symptoms of shortness of breath, chest pain, passing out, and feeling dizzy, the numerous doctors I saw told me that I was most likely depressed, had anxiety, or was just stressed due to being a mother.

A friend of mine recommended a cardiologist in Monterey, CA. He discovered that my heart was enlarged and made arrangements for me to see the pulmonary hypertension (PH) team at Stanford Hospital. I met with Dr Roham Zamanian and the rest of the team and was hospitalized that same day. That was the day my life completely changed. I was no longer a person who thought about living into old age; now I had to think about living as if every day might be my last.

The PH team started me on triple therapies, and I was to titrate rapidly due to the damage done to my heart. Each month that passed I was expecting to feel a difference in my condition. I was no longer passing out, but I still had chest pain and difficulty breathing due to how advanced my PH was. In March of 2012, my PH team started preparing me for a double lung transplant. I did all the necessary tests, but was hesitant because my daughter was just starting kindergarten and I didn't feel ready.

In June I was approached again and told that I needed the transplant if I was to prolong my life, since my body was not reacting to the medications as well as they had hoped. In August of 2012, I was officially added to the lung transplant list. I was scared and didn't feel ready. Dr Zamanian called me that same day and reassured me that I needed to be on the list; then he asked me if I was open to trying a trial medication.

I immediately agreed and started my journey with a clinical trial. Dr Zamanian and Dr Edda Spiekerkoetter were the doctors that I would work with during the trial process. I started taking the trial drug and did regular blood tests to ensure that I was within the range that was required. Within a month I couldn't believe how "normal" I felt. I wasn't winded when climbing stairs, my chest pain was gone, and I felt so much better. Within 2 months' time, I needed to follow up with the lung transplant team; when I described how I was feeling, my transplant doctor put my status on the lung transplant list on hold. I knew I couldn't forget that I had PH, but I felt that I could live a life that was productive for as long as I could.

The trial therapy added to the standard of care gave me 5 years before I needed a heart and double lung transplant. During those 5 years I was able to volunteer at my children's school, enjoy birthday parties with them, and travel in and out of the country, making memories along the way.

Due to the nature of PH, when you start to decline, it can happen fast. In December of 2016, my transplant team encouraged me to start the lung transplant process again. In my mind I was strong and thought I could make it a couple more years without transplantation, but my body was giving out. In January 2017 I was admitted to the hospital at Stanford with a central line infection. In the next few days I started to rapidly decline and was placed on the transplant list on February 14 for a heart and double lung transplant.

My condition improved and I was allowed to go home and wait for the transplant call. I was home for almost 2 months when I started to cough up blood and was taken again to Stanford Hospital. I don't remember what happened during that period; I woke up 5 days later and realized I was connected to the extracorporeal membrane oxygenation machine in the intensive care unit.

As I waited for my transplant, I would think about the extra time I gained with my family due to the trial therapy in addition to excellent care. Those memories brought me a lot of happiness, comfort, and peace. I was transplanted on June 4, 2017, and though I no longer have PH, I hope that my experience with trying the trial drug helps some other PH patients and encourages other to try trial drugs in the future.

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Ask the Expert: A Regulatory Perspective on Clinical Trials for Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a rare, progressive disease. There are 11 drugs available in the United States to treat adult PAH patients; however, all drugs primarily act through vasodilation and have modest effects on clinical endpoints. None of these drugs can claim survival benefit in their product labels. New drugs are needed that target other mechanisms in the disease to have durable benefits for patients. To demonstrate clinical benefit, new drugs are now tested in large, randomized, placebo-controlled trials evaluating their effect to delay clinical worsening, a composite endpoint of morbidity events and death. Efficient clinical trial designs, such as the use of enrichment strategies, that reduce the number of patients and trial duration would be valuable for this disease. It would also be desirable to have new clinical endpoints that measure improvement in quality of life and allow the use of extrapolation strategies to the pediatric population. Academic, industry, and regulatory partnerships are key to advancing therapies for this disease.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is still considered a rare disease for drug development. The Orphan Drug Act (ODA) defines a rare disease as one affecting fewer than 200 000 in the United States.[1] Although the prevalence of PAH is estimated to be around 10 per million in the United States, pulmonary hypertension was given orphan disease status in 1985 when the prevalence of the disease was thought to be <200 000.[2–4] The ODA gives pharmaceutical companies financial incentives to develop drugs to treat rare diseases affecting a limited patient population.[5] The ODA does not, however, relax the criteria for "substantial evidence" needed to demonstrate that the drug is effective in treating a disease. For PAH, evidence of effectiveness has usually been satisfied by a single multicenter, randomized, placebo-controlled clinical trial demonstrating clinical benefit, supported by other studies showing hemodynamic improvement or clinical benefit in other pulmonary hypertension groups. The Food and Drug Administration (FDA) approved the first PAH-specific therapy (epoprostenol) in 1995 and has subsequently approved 10 additional new drugs over the past 2 decades. Most drugs have demonstrated clinical benefit by improving the 6-minute walk distance or, more recently, by decreasing the occurrence of clinical worsening. Although none of these drugs can claim survival benefit in their product labels, survival in patients followed in PAH registries has improved since the availability of these therapies.[4,6] The 5-year survival is 61% compared with 34% in the 1980s.[6,7] Besides the availability of PAH-specific therapies, other possible reasons for the improved survival are lead-time bias due to better awareness of PAH, better clinical management of right ventricular failure, and better outcomes in patients receiving heart-lung transplants.[4,6] Despite the significant progress in treating patients with this rare disease, drug development challenges remain, such as finding drug mechanisms other than vasodilation, improving the efficiency of clinical trials that use time to clinical worsening as their primary endpoint, developing endpoints that reflect benefits in patient symptoms and quality of life, and expanding the number of drugs available to pediatric patients with PAH.

DRUGS TARGETING OTHER MECHANISMS

Patients with PAH exhibit enhanced pulmonary arteriolar contractility, endothelial dysfunction, remodeling and proliferation of endothelial and smooth muscle cells, and thrombosis.[8] The outcome of these physiological changes is partial occlusion of the small pulmonary arteries leading to increased pulmonary vascular resistance (PVR), right heart failure, and death. All approved drugs primarily act through vasodilation, which, considering how small the drug effects are, must be a minor component of the disease. These drugs target 3 key signaling pathways in smooth muscle cells: prostacyclin, nitric oxide, and endothelin (ET) pathways.[9] Prostacyclin analogues (epoprostenol, treprostinil, ilo-

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prost) and receptor agonists (selexipag) increase cyclic adenosine monophosphate concentrations in smooth muscle cells and cause pulmonary vasodilation. The phosphodiesterase-5 inhibitors (sildenafil, tadenafil) and guanylate cyclase stimulators (riociguat) augment nitric oxide-cyclic guanosine monophosphate pathways and promote the vasodilatory and antiproliferative effects of nitric oxide. ET receptor antagonists, which are available as selective for ET_A (ambrisentan) or nonselective for ET_A and ET_B receptors (bosentan, macitentan), decrease ET concentrations and promote relaxation and reduced proliferation of smooth muscle cells. The main disadvantage of the currently available agents is that none directly target the adverse vascular remodeling in the pulmonary vasculature, and most do not improve right ventricular function. New drugs are needed that target other mechanisms in the pathophysiology, such as immune dysfunction, vascular cell proliferation, and right ventricular dysfunction.[4]

Drugs that target vasoconstriction have only modest effects on efficacy endpoints. In Phase 3 trials, most drugs have small increases in 6-minute walk distance (average of +30 m), an improvement (relative to placebo) of only about 10% from baseline and small compared with the day-to-day intrapatient variability. Such improvement may not be easily perceived by patients. Selexipag and macitentan showed 40%–45% reduction in the occurrence of clinical worsening, a composite endpoint of death, hospitalization, and other measures of disease progression, but the benefit was attributed to a reduction in hospitalizations for PAH worsening or other disease progression events.[10,11] Oral treprostinil showed 25% reduction in the occurrence of clinical worsening, which was attributable to a reduction in disease progression events, but not with the other components of the endpoint.[12] Administering a combination of ambrisentan and tadalafil reduced the occurrence of clinical failure by 50% compared to pooled monotherapy in treatment-naïve patients at high risk.[13] None of the drugs tested in large, event-driven trials have demonstrated an improvement in survival.

EFFICIENT CLINICAL TRIAL DESIGNS

Clinical trial designs testing new therapies are now large, placebo-controlled, event-driven trials assessing time to clinical worsening in PAH patients receiving background treatment. Patients need to be followed for 3–5 years to achieve the target number of events for statistical power. One approach to improve the efficiency of these trials is to use enrichment strategies.[14] Prognostic enrichment uses patient characteristics to select a higher-risk study population in which detection of a drug effect is more likely than in an unselected population. Prognostic enrichment does not affect the relative risk reduction but increases the event rate, reducing overall sample size requirements. A recent proof-of-concept study demonstrated the feasibility of using the COMPERA,[15] the French score,[16] or REVEAL[17] risk scales to identify PAH patients who are more likely to experience a clinical worsening event for trial enrichment.[18] When these risk scores were applied retrospectively to the Griphon,[11] Ambition,[13] and Seraphin[10] clinical trials, patient enrichment strategies reduced needed enrollment size and the duration of treatment and observation. An enrichment strategy has many significant patient benefits, such as reducing the duration of treatment with placebo and improving time-to-market for potentially lifesaving medications. The FDA has no reservations about bridging treatment efficacy to lower risk groups because the current understanding of the PAH disease state and pathophysiology supports a treatment effect regardless of a patient's individual risk of morbidity or mortality at baseline.

ENDPOINTS THAT REFLECT PATIENT IMPROVEMENT

Primary efficacy endpoints in pivotal PAH trials have been focused on measurements of exercise function (eg, 6-minute walk distance) or assessments of clinical events (eg, composite of morbidity events and death), but have not focused on measures of patient symptoms and how the symptoms impact quality of life. It is desirable to have a patient-reported outcome (PRO) instrument that measures treatment benefit in patients' symptoms as secondary endpoints in clinical trials. Commonly used quality-of-life measures in PAH trials include the 36-item Medical Outcomes Study Short Form Survey (SF-36 v2)[19] or the Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR)[20] questionnaire, but none of these measures has been used to support a labeling claim. Recently, the Pulmonary Arterial Hypertension-Symptoms and Impact Questionnaire (PAH-SYMPACT) instrument for quantifying PAH symptoms was developed and evaluated as a PRO instrument for PAH patients.[21] The questionnaire measures important, patient-relevant aspects of PAH symptoms and impacts of the symptoms that are not captured by other clinical endpoints. PRO instruments can support a labeling claim; interactions with FDA's Clinical Outcomes Assessment (COA) Staff can assist in developing instruments with a good chance of successfully demonstrating drug effects.[22] The FDA lists information about submissions to the COA Qualification Program, including FDA's decision to accept or not accept the submission.[23]

PREDICTIVE BIOMARKERS AND SURROGATE ENDPOINTS

PAH is a disease that lacks validated surrogate endpoints appropriate for approval. A surrogate endpoint is expected to predict clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence and is used in clinical trials as a substitute for a direct measure of how a patient feels, functions, or survives.[24] The FDA has used PVR as a surrogate endpoint under specific scenarios for drugs that have been approved for the treatment of PAH. The FDA evaluated the relationship between change from baseline in PVR and 6-minute walk distance using pooled patient-level data from 2028 adults with PAH in controlled, clinical trials.[25] The estimated slope [0.055 m/dyne·s/cm^5 (95% CI = 0.62, 0.047)] was consistent in magnitude across 4 drug classes and 9 individual drugs. The FDA used the relationship to extrapolate the efficacy from

adults to children using PVR to approve bosentan in pediatric PAH patients, where PVR was determined during right heart catheterization.[26] This approach cannot be used for other drugs because of the view that right heart catheterization poses more than minimal risk for pediatric patients; therefore, assessment of PVR as obtained through the use of right heart catheterization is no longer considered appropriate in pediatric trials.[27] In adults, PVR has been used as a primary endpoint in clinical trials testing the efficacy of combination therapy of 2 PAH drugs and to assess whether a new therapy has a sustained effect on PVR after the drug was discontinued. As drugs targeting new pathophysiology processes in PAH enter clinical development, the endpoints should be tailored to the disease biology and anticipated mechanistic effects, thereby allowing for potential regulatory consideration of novel biomarkers.[28]

DRUGS TO TREAT PEDIATRIC PAH

Although 11 drugs have been approved in the United States for the treatment of PAH in adults, to date only bosentan has been approved for the treatment of PAH in children. The FDA's approach using PVR as a surrogate endpoint to bridge dose response with clinical efficacy cannot be generalized to other drugs because the routine use of serial right heart catheterizations in clinical trials is now considered unethical in children. There is widespread recognition that treatments are needed for children with PAH, but it has been difficult to conduct trials in this population.[29] One reason that has been cited is the lack of clinical equipoise once a new treatment is approved for adults and used extensively off label in children. Moreover, clinical practice guidelines for pediatric PAH recommend similar treatment strategies that are used in adults despite the lack of randomized clinical trials of the same therapies in children.[30] Another challenge has been identifying feasible and reliable endpoints for demonstrating efficacy in children. The 6-minute walk test has been used in most drug development programs to establish the efficacy of new therapies for PAH in adults. The 6-minute walk test is not appropriate for all children with PAH for reasons of reliability in young children (less than 6 years) and those with developmental impairment.[27,31] Clinical trials using time to clinical worsening endpoints may not be feasible in pediatric trials because they generally require large trials and long duration of follow-up to observe events. Extrapolating the effectiveness of approved PAH treatments for adults to the pediatric population will require the development of noninvasive predictive biomarkers that are as robust as PVR. Therefore, novel approaches to both trial design and endpoints are needed to evaluate the efficacy and safety of PAH treatments in children. The FDA is open to discussing alternative pathways, novel endpoints, and novel trial designs with sponsors who are developing treatments for pediatric patients with PAH.

CONCLUSION

Although the FDA will still approve nonspecific vasodilators for PAH, and such drugs remain in development, particularly for less well-studied forms of PAH, the era of the nonspecific vasodilator is ending. Antiproliferative therapy seems likely to have the potential to achieve larger, more durable benefits.

The FDA applied a fairly low standard for approval based on improvements in exercise capacity that were likely too small to be considered clearly clinically relevant. This, too, is changing, and more recent approvals have incorporated a clinical worsening endpoint for which there is no lower bound for clinical relevance.

Academic, industry, and regulatory partnerships are key to making the best use of available data to inform efficient trial design for new drugs in adults and to bridge existing therapy to pediatric populations.

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