From basic scientific research to the development of new drugs for pulmonary arterial hypertension: insights from activin-targeting agents

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Shareable abstract (@ERSpublications)
The combination of human sample data, clinical data, and preclinical research enhances our understanding of vascular remodelling mechanisms. This integrated approach fosters synergy, validates hypotheses, and accelerates treatment innovation. https://bit.ly/3zY2eLr

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Abstract

Pulmonary arterial hypertension (PAH) is a severe disorder of the pulmonary vasculature leading to right ventricular failure. This pulmonary vascular remodelling leads to increased pulmonary vascular resistance and high pulmonary arterial pressures. Despite the development of new therapies, many patients continue to experience significant morbidity and mortality. This review offers a comprehensive overview of the current understanding of PAH pathophysiology, with a focus on key mechanisms that contribute to pulmonary endothelial cell dysfunction and the pathological accumulation of pulmonary artery smooth muscle cells, mesenchymal cells and inflammatory cells in the walls of remodelled small pulmonary vessels, three processes central to the progression of PAH. In particular, it highlights recent developments in targeting the activin signalling pathway, a novel therapeutic approach that shows promise in modulating these pathological processes. The review also addresses the ongoing challenges in translating preclinical findings into effective clinical treatments, emphasising the importance of integrating human data with preclinical models and adopting innovative strategies to bridge the gap between research and clinical practice.

Introduction

In recent years, significant strides have been made in the management of pulmonary arterial hypertension (PAH), a rare and severe pulmonary cardiovascular condition defined by a mean pulmonary arterial pressure (mPAP) >20 mmHg at rest, with a pulmonary vascular resistance (PVR) >2 Wood units and a pulmonary artery wedge pressure ⩽15 mmHg [1, 2]. This increase in PVR and pulmonary pressures is explained by the onset of intense progressive remodelling of the distal pulmonary blood vessels, driven by three key forces: dysfunction of pulmonary endothelial cells (ECs), dysfunction of smooth muscle and the adventitia, and persistent chronic inflammation, which is notably associated with the complex recruitment of inflammatory and immune cells within the lungs (figure 1) [3]. Over the past three decades, the development and commercialisation of over 12 new drugs have markedly improved patients' quality of life, reduced short-term mortality risk and slowed disease progression. Despite these advancements, many patients continue to face substantial challenges, including reduced exercise capacity, lower quality of life, and a concerning prognosis with insufficient survival rates [4].

Recent innovations, such as sotatercept (a soluble receptor combining the Fc domain of human IgG with the extracellular domain of human ACTRIIA), have expanded treatment options, primarily targeting the endothelin (ET)-1, nitric oxide (NO), and prostacyclin (PGI_2) pathways [5, 6]. However, despite numerous new therapeutic targets emerging from preclinical studies, only a few have advanced to clinical trials. The

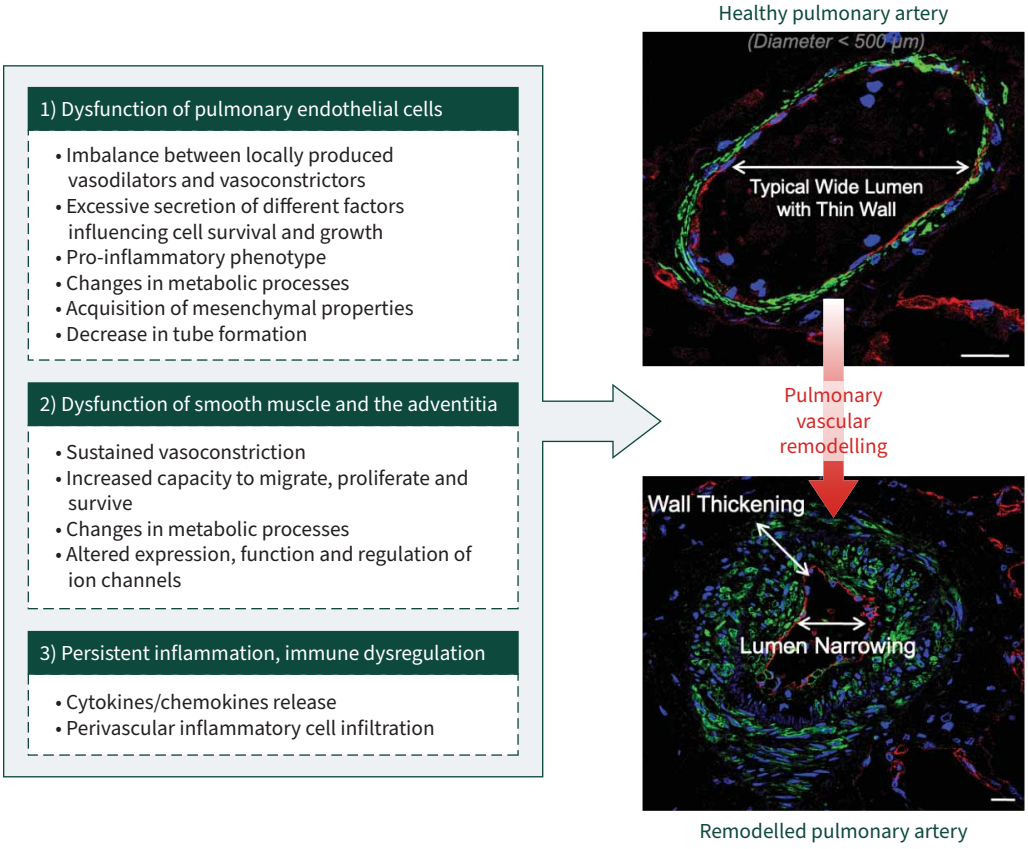

FIGURE 1 The three driving forces of pulmonary vascular remodelling associated with pulmonary arterial hypertension. The pulmonary vascular smooth muscle was immunolabelled in green using an anti-alpha smooth muscle actin antibody, the endothelium was labelled in red with a CD31 antibody, and the nuclei were stained in blue using DAPI (4′,6-diamidino-2-phenylindole). Scale bar: 50 µm.

path from drug discovery to commercialisation is complex and costly, with clinical trials demanding significant financial investment and long-term commitment from patients. Consequently, the pool of patients available for testing new therapies is limited. Moreover, many innovative molecules do not always deliver the anticipated benefits in patients, a challenge that is not unique to PAH. The translation of scientific discoveries into effective treatments remains a widespread issue across various diseases. Improving the accuracy of preclinical studies and optimising drug development through more robust experimental data is crucial. This review explores the obstacles, opportunities and strategies for enhancing drug development for PAH, emphasising the need for more rigorous and innovative approaches to provide better therapeutic options and ultimately improve patient outcomes.

Current insights into the pathophysiology of PAH

The pathogenesis of PAH involves a complex, multifactorial process driven by a dynamic network of molecular players [3]. The distal pulmonary vasculature undergoes sequential remodelling, characterised by a range of alterations that lead to the progressive muscularisation of the walls of small- and medium-sized precapillary pulmonary arteries. This remodelling results in significant narrowing of the pulmonary vascular lumen, which restricts normal blood flow, increases PVR and elevates the mPAP above normal levels (typically to ~14±3 mmHg) [2]. The increase in PVR is further exacerbated by the loss or pruning of the distal part of the pulmonary circulation, leading to even higher PVR levels. This pulmonary vascular remodelling involves changes in all three layers of the vascular wall, driven by the accumulation of pulmonary ECs, pulmonary artery smooth muscle cells (PA-SMCs), and various mesenchymal cell types, including fibroblasts, myofibroblasts and pericytes. These cells interact with a variety of inflammatory and immune cells both within and around the pulmonary arterial wall. In addition, this process is associated with extracellular matrix (ECM) deposition and degradation, which contributes to the progressive narrowing of the pulmonary vascular lumen and, in some cases, complete obstruction.

Recent advances in our understanding of PAH pathophysiology highlight the complexity and multiplicity of mechanisms involved in vascular remodelling [3]. Key molecular players include various vasoactive and proliferative factors such as ET-1, NO, PGI_2, angiotensin-II, serotonin, platelet-derived growth factor (PDGF), beta-nerve growth factor (β-NGF), fibroblast growth factor-2, epidermal growth factor and leptin. Pro-inflammatory mediators, such as interleukin (IL)-1, IL-6, CCL2 and CXCL12, and macrophage migration inhibitory factor (MIF) also play critical roles [7]. In addition, significant molecular actors include various members of the bone morphogenetic protein (BMP)/transforming growth factor (TGF)-β family, such as BMPRII, endoglin and ALK1, as well as defects in ion channels (*e.g.* KCNK3/TASK1), and alterations in metabolic pathways and key enzymes, transcription factors and epigenetic regulators (*e.g.* FOXF1, FOXO1, hypoxia-inducible factor, Notch and peroxisome proliferator-activated receptor-γ) [3].

Alterations affecting the BMP/TGF-β signalling pathway are central to the molecular network involved in pulmonary vascular remodelling [8]. These alterations influence not only the onset but also the progression of the disease. Notably, ∼70% of heritable PAH and 15–40% of idiopathic PAH cases develop in the context of germline autosomal dominant mutations in the *BMPR2* gene [9, 10]. BMPRII, a TGF-β family receptor signalling through Smad1/5/8, is highly expressed in pulmonary vascular cells, including ECs, PA-SMCs and inflammatory cells. However, the low penetrance (∼20%) of *BMPR2* mutations suggests that additional triggers are necessary for PAH development [10]. Interestingly, women with *BMPR2* mutations are more likely to develop PAH, resulting in a higher prevalence of the condition in females compared with males, estimated at about 42% *versus* 14% [9]. This ratio is consistent with findings from the prospective follow-up study of asymptomatic *BMPR2* mutation carriers, DELPHI-2, which reported an annual incidence of 0.99% in men and 3.5% in women [11]. Thus, there is a strong link between the dysfunction of the BMPRII pathway and the risk of developing the disease. However, its role in disease progression appears less significant than defects in other BMP/TGF-β pathway members, such as those in the activin signalling pathway (figure 2). Research into PAH has long been hindered by the complexity of BMP/TGF-β family pathways, which depend not only on the abundance of BMPRII but also on various other receptors that form tetramers to determine the specific cellular response at any given time. These include ACTRIIA, ACTRIIB, TGFβRII and type 1 receptors, also known as ALKs, of which there are seven (ALK1–ALK7) [8]. The composition of these tetramers is crucial and they bind to different ligands, activating the Smad1/5/8 and/or Smad2/3 pathways to varying extents and durations, often in association with non-Smad pathways [8]. Overactivation of the activin pathway is one potential mechanism that contributes to the excessive phospho-Smad2/3 levels observed in the lungs of PAH patients [12]. This is particularly notable given that elevated serum levels of activin A and follistatin-like 3 (FSTL3) have been identified as predictive markers for adverse outcomes in PAH, including death or the necessity for lung transplantation [12]. Furthermore, transgenic mice that overexpress *INHBA*, the gene encoding the *inhibin-βA* subunit of activin A, show exacerbation of experimental pulmonary hypertension (PH), while mice with reduced expression of activin A are protected against the pulmonary vascular remodelling induced by chronic hypoxia [13]. However, other factors, including TGF-β, connective tissue growth factor and potentially other molecules, may also play a role in this increased Smad2/3 signalling, either directly or indirectly [8]. Further research is necessary to fully understand these mechanisms and their implications for PAH progression.

Despite the complexity of the BMP/TGF-β pathways, with their structural similarities among ligands and cell-specific functions, researchers have explored various strategies to mitigate the phosphorylation of Smad2/3 [14–19]. Approaches have included heterodimeric and homodimeric ligand traps, neutralising antibodies, recombinant proteins, specific small molecules, antisense drugs, and gene therapy. Among these, sotatercept stands out as a novel recombinant fusion protein that functions as a ligand trap for activins and various BMP/growth and differentiation factor (GDF) ligands, such as GDF-11, GDF-8, BMP-10 and BMP-9 [20]. Clinical trials have demonstrated its efficacy in reducing mPAP and PVR, improving 6-min walk distance and addressing other secondary end-points, making it a promising treatment for PAH [5, 6]. Nonetheless, careful monitoring is required due to the potential side-effects of sotatercept, which may include elevated haemoglobin levels, thrombocytopenia, telangiectasia and bleeding complications, such as epistaxis and, on rare occasions, gastrointestinal bleeding [21, 22]. The drug has received approval for PAH treatment from both US and European Union regulatory agencies, representing a notable advancement in biopharmaceutical therapies for this disease [23]. Building on this foundation, other agents targeting activins are currently under development, such as modified soluble ACTRIIB receptors like KER-012 and HS135, paving the way for new therapeutic avenues.

This evolving understanding underscores the complexity of PAH and highlights the need for continued research to understand how these activin-targeting agents work and modulate the molecular and cellular mechanisms driving the progression of this life-threatening condition.

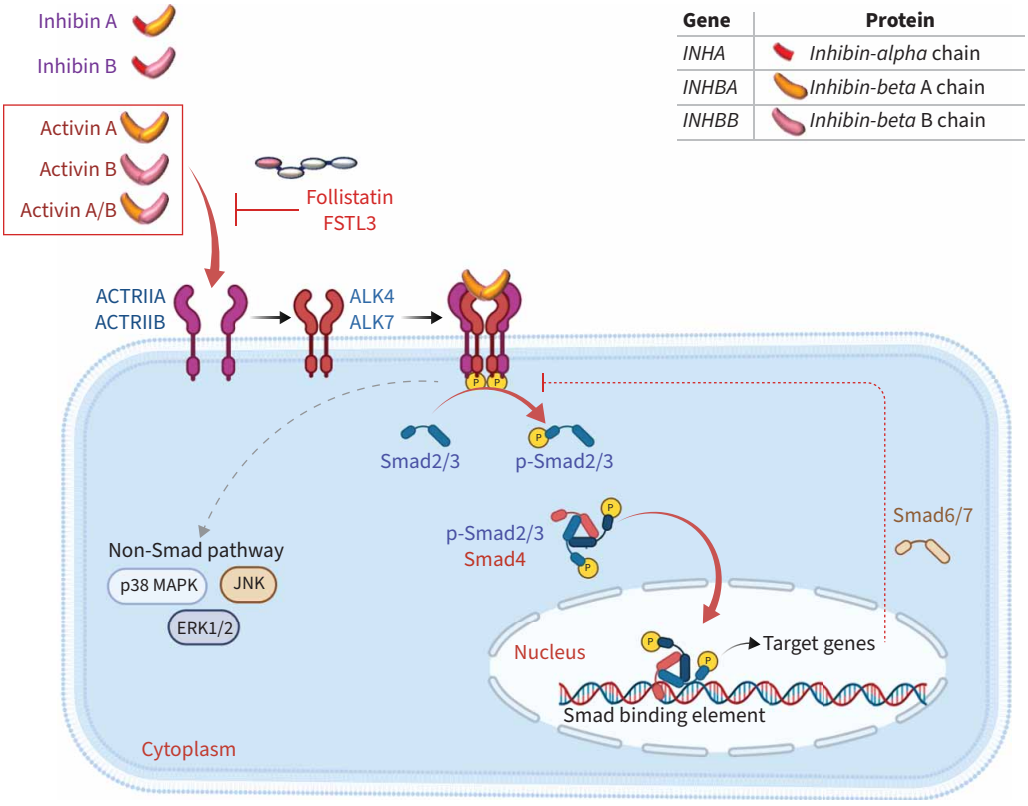

FIGURE 2 The activin signalling pathway. The activin signalling pathway is a subset of the bone morphogenetic protein (BMP)/transforming growth factor (TGF)-β pathway. Activins are dimers composed of two *inhibin-beta* chains (*inhibin-βA* and *-βB*) that have a high affinity for the type 2 receptors, ACTRIIA and ACTRIIB. Upon binding of activin A, activin B or activin A/B, these receptors recruit a type 1 receptor (ALK4 or ALK7). This recruitment leads to the phosphorylation of Smad2/3, which occurs to varying degrees and durations. The phosphorylated Smad2/3 then translocates to the nucleus in complex with Smad4 to regulate the expression of target genes involved in various cellular functions. These cellular functions include the modulation of endothelial cell activities such as tube formation, the regulation of smooth muscle cell contractility, and the recruitment and activation of inflammatory and immune cells. The pathway is tightly regulated at multiple levels, starting from the formation of activin dimers. The *inhibin-β* chains can also bind to an *inhibin-α* chain to form inhibin A and inhibin B, which do not induce the phosphorylation of Smad2/3. Furthermore, the binding of activin A, B, or A/B dimers can be inhibited by follistatin and follistatin-like 3 (FSTL3). Figure created with BioRender.

How to improve our understanding of the pathophysiological mechanisms of PAH and enhance clinical translation

To advance our understanding of PAH pathophysiology, it is essential to rely on reliable, robust and reproducible research data. Ideally, these data should come directly from well-phenotyped PAH patients or their samples and tissues. Integrating these human observations with preclinical data is essential for progress (figure 3).

Human observations obtained directly from patients, whether biological samples, clinical data, imaging studies or tissues, provide an invaluable foundation for research. This information helps to better understand PAH's specific characteristics and interindividual variability. However, these data are most useful when combined with preclinical data. Preclinical research plays a complementary role by offering a controlled environment in which to explore underlying pathophysiological molecular mechanisms, determine whether observed alterations are causes or consequences, and test hypotheses. For example, in the context of new approaches targeting the activin pathway, it would be beneficial to investigate whether responses to these interventions can be predicted by circulating biomarkers such as activin A, FSTL3, or other key cytokines and chemokines known to play a role in PAH (*e.g.* IL-6, MIF, β-NGF, CXCL9 and TRAIL).

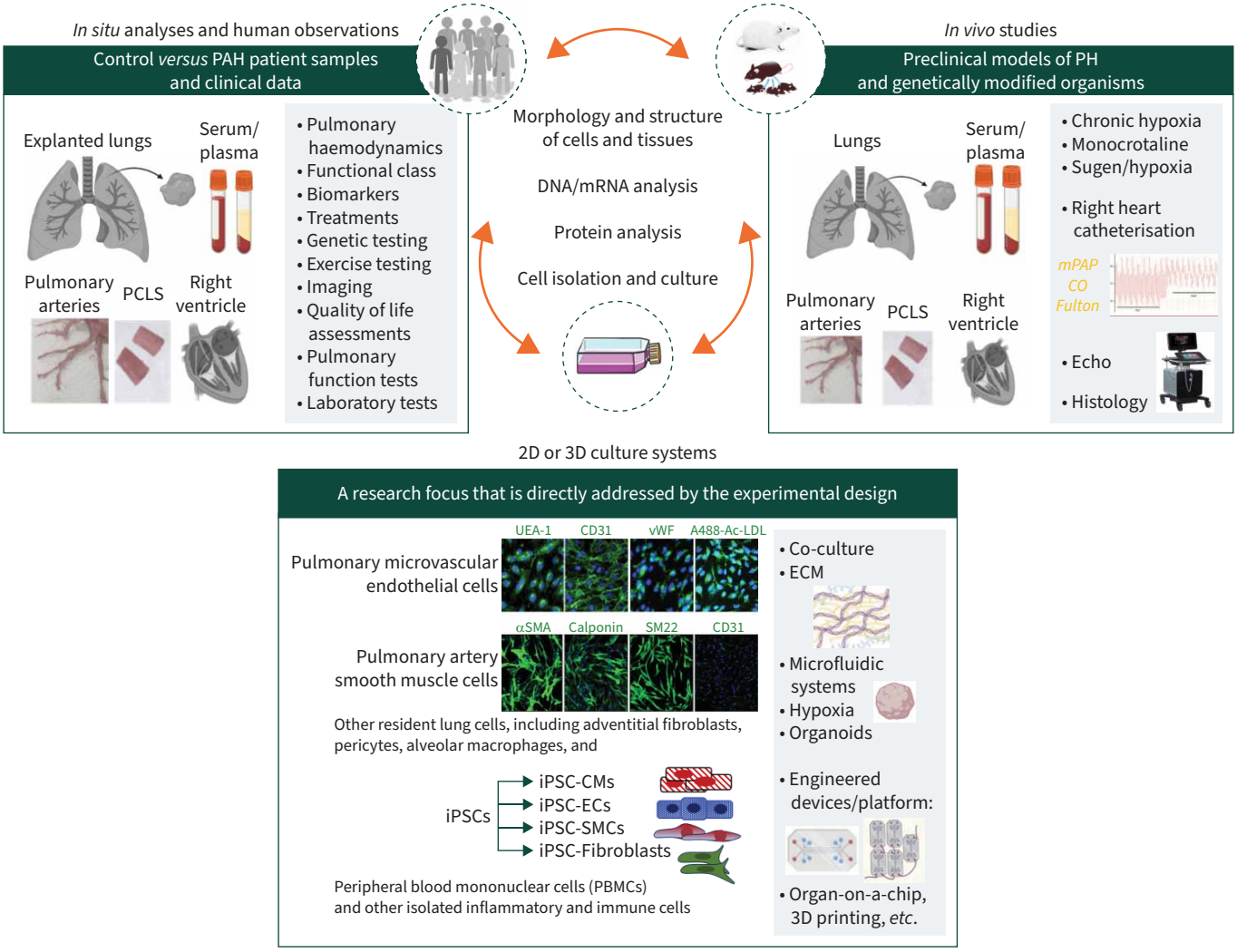

FIGURE 3 Integration of human and preclinical research to advance pulmonary arterial hypertension (PAH) understanding and treatment. This schematic illustrates how merging human data (*e.g.* clinical samples, imaging) with preclinical research (*e.g.* animal models, cell cultures, bioengineered systems) can deepen our understanding of PAH pathophysiology. It highlights the synergy between these approaches in validating research hypotheses and developing innovative treatments. CM: cardiomyocyte; CO: cardiac output; EC: endothelial cell; ECM: extracellular matrix; Echo: echocardiography; iPSC: induced pluripotent stem cell; mPAP: mean pulmonary artery pressure; PCLS: precision-cut lung slices; PH: pulmonary hypertension; SMC: smooth muscle cell.

Preclinical studies can involve well-characterised animal models to study pulmonary vascular remodelling, simulate PH progression, and evaluate the efficacy of new interventions. In addition, cell cultures in both two (2D) and three dimensions (3D), such as primary pulmonary vascular cell cultures, offer simplified yet relevant models for in-depth exploration of cellular and molecular mechanisms. *Ex vivo* experiments, including precision-cut lung slices and bioengineered models like artery-on-a-chip, provide opportunities to study tissue responses in environments that more closely mimic physiological conditions. For instance, using these advanced models could offer deeper insights into how activin signalling contributes to pulmonary vascular remodelling and interacts with other critical signalling pathways, such as PDGF, vascular endothelial growth factor (VEGF), β-NGF, CXCL12 or IL-6. While the activin pathway is known for its role in modulating the contractile phenotype of PA-SMCs [19] and influencing certain endothelial functions *in vitro* [13], its precise effects on the responses of resident cells and inflammatory cells to growth factors or other BMP/TGF-β family members remain poorly understood. Furthermore, exploring how activin affects inflammatory cell behaviour and cytokine profiles could shed new light on its role in disease progression. A recent study found significant alterations in proteins related to inflammation, immune activation, oxidative stress, cardiovascular integrity, lipid metabolism, amino acid metabolism, and cell cycle regulation in patients treated with sotatercept compared to those receiving a placebo [20]. These

findings underscore the need for further investigation into the intricate roles of activin in pulmonary vascular dynamics.

Even with an integrated approach, significant progress cannot be achieved without clearly defined research questions to choose the best experimental strategies. Careful interpretation of data is essential for reaching reliable conclusions. Therefore, the research approach must be adapted according to the type of research conducted. Exploratory research aimed at understanding pathophysiological processes and identifying key signalling pathways will differ from confirmatory investigations, which provide detailed and reproducible information on drug dosing, toxicity levels and modes of action. Although the objectives differ, both types of research are complementary and crucial for advancing PAH understanding and treatment.

In situ *analyses and human observations: a key element*

Access to high-quality, well-phenotyped samples from PAH patients, as well as appropriate controls, is invaluable for advancing our understanding of PAH and validating emerging hypotheses about the cellular and molecular processes involved in the disease's development and progression. These samples may include lung and cardiac tissue fragments, serum, plasma, isolated blood cells, bronchoalveolar lavages, exhaled air, urine and other specimens. Ensuring that PAH diagnoses are fully validated according to current guidelines before using these samples is essential. Moreover, control samples should be age- and gender-matched and, ideally, matched for underlying conditions (*e.g.* scleroderma patients without PAH *versus* those with scleroderma-associated PAH). When working with a limited number of biological samples, caution must be exercised in interpreting correlations between experimental data (*e.g.* molecular, genetic) and clinical data (*e.g.* pulmonary haemodynamics, imaging, exercise testing). However, clinical data are crucial for confirming the suitability of samples used in experiments. For larger sample sizes and depending on the type of samples and studies planned, long-term follow-up and independent validation cohorts should be considered, particularly for studies aimed at identifying circulating biomarkers. Indeed, although several potential biomarkers have been identified, such as activin A, β-NGF, CXCL9, CRIM1, endoglin, FSTL3, GF, netrin-4, neutrophil elastase, PXDN, PLAUR, PRDX4, SVEP1, SPON1, TSP2 and TRAIL, the field still lacks reliable and easily measurable circulating biomarkers [12, 24–27]. The development of one or more biomarker panels is crucial for enhancing patient stratification and guiding treatment choices, particularly for predicting responses to therapies.

Significant progress has been made through the histological analysis of explanted lungs obtained during lung transplants in PAH patients. Advanced analytical techniques, such as spatial transcriptomics and synchrotron-based imaging, offer significant potential to enhance our understanding of PAH. When applied to high-quality samples by domain experts, these technologies could provide deeper insights into subtle variations in pulmonary vascular remodelling among patients with or without predisposing mutations. In addition, they could illuminate the complex relationships recently identified between the pulmonary and systemic circulatory systems, as well as the interactions among pulmonary arteries, veins and lymphatic networks. These human samples could also aid in precisely mapping the BMP/TGF-β pathways, allowing for the direct identification of alterations within the tissues.

Studies often use cells from explanted lungs obtained during lung transplants in PAH patients, compared to control lung tissues generally taken from localised tumours or during lobectomies. When using lung tissue resected for cancer, samples should be taken from areas sufficiently distal from the tumour to avoid significant phenotypic and genotypic influences. It is crucial that tissues are selected and processed consistently with the PAH samples to ensure homogeneous and reliable results. Cells isolated from PAH patient lungs cannot be compared to commercially available cells due to differences in isolation techniques, which can introduce significant bias.

Interpreting the data also requires careful consideration of several factors. First, the samples (tissues or cells) are typically from patients at an advanced stage of the disease. By the time PAH is diagnosed, a significant portion of the pulmonary circulation is already compromised, contributing to elevated mPAP. PAH patients often receive various treatments and have comorbidities, which can influence the collected data. Proteins and signalling pathways observed in advanced-stage tissues may be altered by these treatments or reflect chronic disease effects, rather than the initial mechanisms of PAH development or progression. Obtaining lungs from untreated PAH patients at an early stage or conducting longitudinal assessments is nearly impossible due to biopsy risks.

Finally, using human tissues involves significant ethical responsibilities. Securing ethical approval, ensuring informed consent, and maintaining patient confidentiality and anonymity are crucial. Researchers should ensure that all necessary ethical approvals are obtained and adhered to.

The role of in vitro *studies and their valuable contributions to research*

Conventional 2D and 3D culture systems, microfluidic *in vitro* systems, and cell co-cultures have significantly advanced translational research on PAH and biomedical discovery. These models have demonstrated the ability to preserve abnormal cellular phenotypes outside their natural environments, especially in early passages of primary cultures. For instance, pulmonary ECs from PAH patients exhibit a pro-inflammatory phenotype and a reduced capacity to form tubular structures [28, 29], while PA-SMCs show imbalances in proliferation/apoptosis and various metabolic alterations [3]. To further this research, it would be valuable to investigate in detail the role of activins and other BMP and GDF ligands in modulating responses to key factors involved in pulmonary vascular remodelling in PAH using these different *in vitro* tools. Such studies could clarify whether this pathway counteracts or amplifies the effects of certain growth or differentiating factors.

These systems allow examination of how different types of pulmonary vascular cells from patients with severe PAH behave outside their pathological microenvironment. *In vitro* assays facilitate the evaluation of cellular responses such as proliferation, survival, migration and tube formation in the presence of agonists or antagonists. Strategies involving the knockdown or overexpression of molecular actors (small interfering RNA (siRNA), short hairpin RNA (shRNA), adeno-associated virus (AAV) or expression plasmids) can also be implemented to refine these evaluations. Manipulating these primary cells *in vitro* using techniques like CRISPR/Cas9 or viral infection enhances their versatility. The integration of new technologies, such as induced pluripotent stem cells (iPSCs), further enriches research possibilities. iPSCs, derived from somatic cells of patients, whether they harbour mutations or not, can differentiate into various cell types, including ECs, smooth muscle cells, pericytes, fibroblasts, cardiomyocytes and myeloid cells. 2D or 3D culture models can also be employed to develop high-throughput screening systems or to identify therapeutic agents. For PAH, bioengineered models such as “artery-on-a-chip” and pulmonary microvascular networks on a chip are considered particularly relevant. Future research could benefit from focusing on “vessel-on-a-chip” models to address the complexities of lung vascular research.

To enhance the translational efficiency of *in vitro* studies, it is crucial to validate results with *in situ* observations and carefully select the most relevant cell types and techniques. Clearly defining the research problem is essential for choosing the appropriate cells and techniques. In addition, it is important to maintain the expression of characteristic markers in long-term cultures and to ensure that identical protocols are used for isolating and culturing “control” and “PAH” cells. Given that primary cell cultures may lose phenotypic properties with passaging and remain fragile due to chronic stress, characterisation and purity assessments should be conducted before use.

Particular attention should also be given to culture protocols, including whether experiments are conducted with synchronised cells, the type of support used, the presence of specific coatings with matrix proteins, the rigidity of the support, and the composition of the culture media (with or without additives such as growth factors, serum percentage, *etc.*). These details significantly influence the data collected, making precise documentation essential for accurate interpretation of results.

Incorporating factors such as inflammatory mediators, shear stress and hypoxia into *in vitro* models can improve their relevance to PAH conditions. 3D cell culture models, where cells are grown in ECM gels, facilitate the study of cell–matrix interactions and vascular structure formation. Additionally, co-culture systems and organ-on-chip technologies offer opportunities for more complex and realistic experiments.

Nevertheless, even with the best *in vitro* strategies, it is impossible to fully replicate the characteristics of living lungs affected by PAH. This underscores the need to combine these *in vitro* studies with *in situ* observations and animal models to validate *in vitro* findings and ensure effective translational research. Moreover, *in vitro* studies may not always be suitable or practical for certain specific experiments, highlighting the importance of considering their strengths and limitations.

In vivo *preclinical research: from simple demonstration to proof of concept*

An ideal model of PH would accurately replicate the genetic basis, anatomy and physiology of the human condition, reproduce similar phenotypes, and predict the clinical efficacy of drugs. However, as the term “model” implies, animal models can only represent a component or aspect of a disease process, and no single model fully mimics human PAH. Moreover, disease induction, which often requires the use of toxins with or without chronic hypoxia exposure, is frequently associated with alterations in other organs. Consequently, these animal models are often referred to as having PH rather than PAH. They should be considered as models of pulmonary vascular remodelling more broadly. Despite these limitations, animal

models remain crucial for advancing scientific knowledge, validating new targets or treatments, and providing insights into disease mechanisms.

In preclinical research, it is essential to define the research problem as precisely as possible and to thoroughly evaluate the strengths and weaknesses of each available PH model. Fortunately, the field of PAH benefits from several distinct but complementary animal models, which has significantly contributed to drug development. Among the most recognised and widely used animal models of PH are chronic hypoxia (CHx), monocrotaline (MCT), and the combination of chronic hypoxia with the VEGF receptor inhibitor SU-5416, also known as sugen (SuHx) (figure 4).

Each model provides unique insights into disease progression and therapeutic potential, making it essential to combine them to test hypotheses or evaluate potential therapeutic agents. The CHx model involves exposing animals to low oxygen levels (10% oxygen) over extended periods (usually 3 or 4 weeks), inducing pulmonary vascular remodelling considered adaptive, as it completely disappears within a few weeks (2–3 weeks) after returning to normoxic conditions (21% oxygen). This type of remodelling reflects the plasticity of the pulmonary circulation, which varies by species and strain. In contrast, the MCT model,

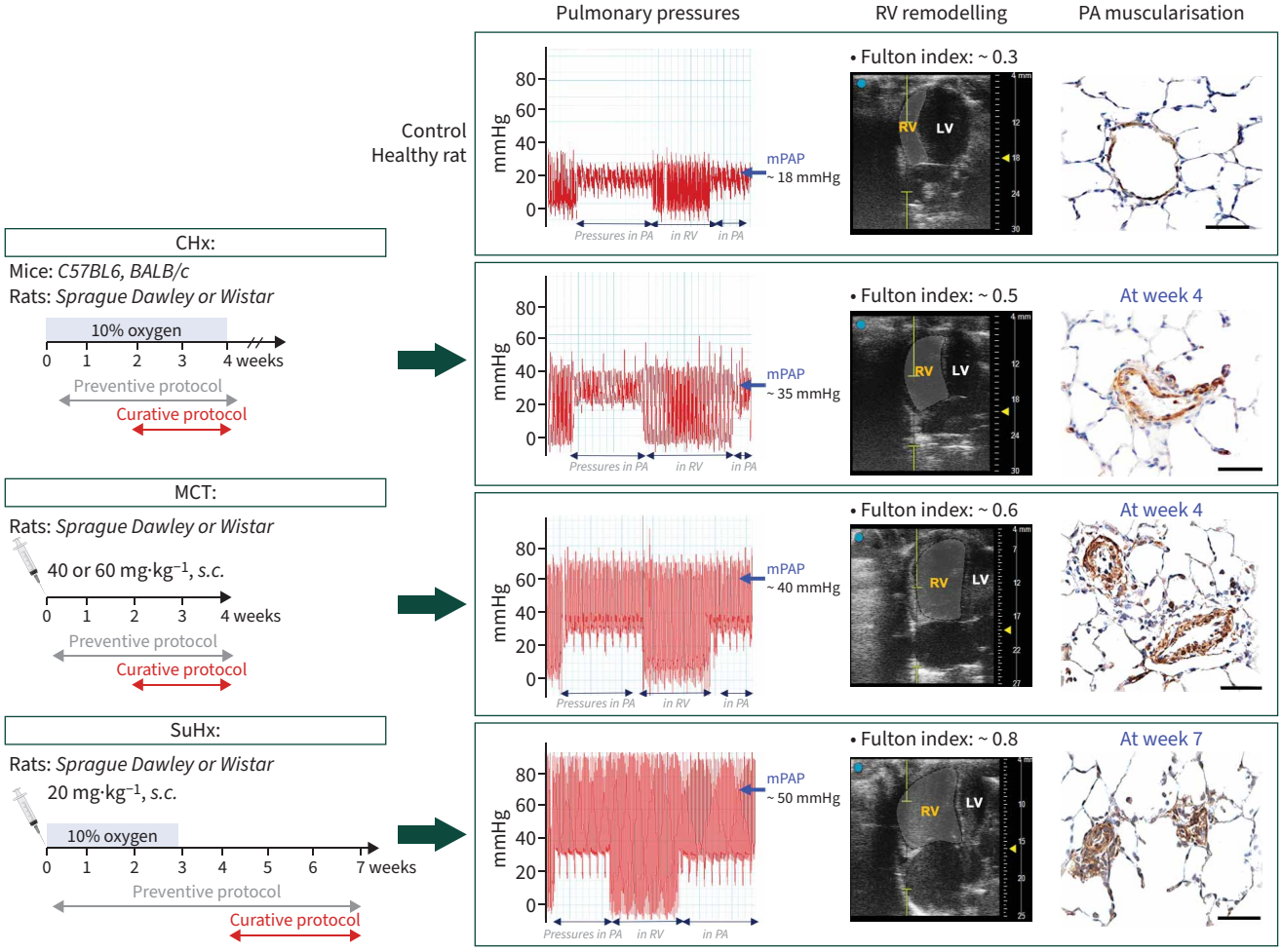

FIGURE 4 The three most commonly used preclinical models of pulmonary hypertension for studying pulmonary vascular remodelling: chronic hypoxia (CHx) in mice and rats, and monocrotaline (MCT) and sugen/hypoxia (SuHx) in rats. The left side of the diagram illustrates commonly used experimental protocols to explore molecular mechanisms and evaluate the efficacy of potential drugs in both preventive and curative approaches. On the right side of the diagram, specific examples are provided, including pulmonary haemodynamic measurements obtained through right heart catheterisation, assessments of right ventricle (RV) remodelling using the Fulton index and two-dimensional echocardiography visualisation of the four cardiac chambers, and images showing small pulmonary artery (PA) muscularisation with immunostaining for α-smooth muscle actin (stained in brown). These data were obtained in Sprague Dawley rats weighing 100 g at the start of each protocol. mPAP: mean pulmonary artery pressure; LV: left ventricle.

which involves administering a toxic alkylating agent, induces a more inflammatory response with significant mortality due to widespread organ damage, including to vital organs. This high mortality rate, which can reach 75% at 4 weeks, is an important parameter that must be carefully considered in study design and presented during data reporting. The pulmonary vascular remodelling observed in the MCT model is severe but is also associated with conditions, such as pulmonary interstitial oedema, myocarditis and hepatic veno-occlusive disease, which are not characteristic of human PAH. The SuHx model leads to more pronounced vascular remodelling, particularly in small-diameter pulmonary vessels, and is less inflammatory than the MCT model. Unlike the MCT model, SuHx does not typically result in high mortality, offering a different profile from human PAH. As with the CHx and MCT models, factors such as body weight, which often correlates with age in rodents, species, strain and sex can significantly impact disease severity. These parameters must be carefully controlled, and experimental groups should be comparable to interpret the data accurately. Despite the limitations inherent to each model, combining CHx, MCT and SuHx provides a comprehensive overview of the various molecular mechanisms driving pulmonary vascular remodelling. This integrative approach is particularly valuable in confirmatory studies and proof-of-concept research. By leveraging the diverse insights provided by these models, researchers can more effectively validate new therapeutic agents and assess their potential effects in the context of existing PAH treatments. Combining models also helps identify additive or synergistic effects when new drugs are tested in combination with existing US Food and Drug Administration-approved PAH medications.

Preclinical research on the development of activin-targeting agents has integrated traditional studies, using well-established animal models of PH [14, 19], with investigations involving transgenic mice that have altered activin production [13]. In addition, this research has leveraged insights from studies on other members of the BMP/TGF-β pathway, including ALK1, TGF-β, BMP-9 and BMP-10 [16–18, 30–34]. It has been shown that a rodent-adapted form of sotatercept can mitigate pulmonary vascular remodelling induced by MCT and SuHx, with the effects partly mediated by reduced inflammatory infiltration [14, 19]. Furthermore, these studies have demonstrated that the sotatercept-like agent exhibits multiple beneficial effects, a finding that has been clearly confirmed in patients treated with this agent, who not only experience anti-inflammatory benefits but also see direct and indirect improvements in the cardiovascular system and in the metabolism of amino acids and lipids [20]. It is now crucial to gain a detailed understanding of the mechanism of action and to determine whether we can identify even more effective agents to combat pulmonary vascular remodelling. In this context, it would be valuable to assess whether these agents can truly reverse the remodelling of pulmonary vessels, including those that have lost their lumen, or if they offer protection against the loss or further deterioration of small vessel [35].

To accurately assess the impact of treatments and understand disease mechanisms, preclinical studies should include a range of standard parameters and techniques. Right heart catheterisation using the closed chest technique remains the gold standard for precisely measuring mPAP in rats, and right ventricular systolic pressure in mice. However, a comprehensive evaluation of PH severity also requires additional assessments. These include evaluating right ventricular hypertrophy using the Fulton index and analysing pulmonary artery muscularisation by measuring the percentage of muscularised vessels and wall thickness. Furthermore, secondary parameters such as cardiac output and systemic blood pressure are crucial for a complete understanding of haemodynamics. Noninvasive techniques such as echocardiography, cardiac computed tomography, magnetic resonance imaging, lung function tests and exercise tests can provide additional valuable data for a more thorough evaluation.

In addition to the most commonly used animal models, there are alternative models such as the rat treated with MCT and subjected to pneumonectomy, either in adulthood or at a young age, where the severity of pulmonary vascular remodelling is even more pronounced. Other models include the left-to-right shunt in piglets and calves, schistosomiasis-induced PH, and simian–human immunodeficiency virus (SHIV)-nef-infected macaques. Some models even combine multiple factors, such as MCT with CHx or surgery, to better reflect the complexity of the disease. Future preclinical research should focus on developing more sophisticated animal models that capture the disease heterogeneity. Regardless of the model used, it is crucial to ensure robust haemodynamic characterisation to obtain reliable data. The impact of anaesthesia duration on data should also be considered. Therefore, it is advisable to collaborate with experts who have experience with these models, as even seemingly simple models can yield unreliable data if not properly managed. Furthermore, advanced imaging techniques can provide more precise and dynamic insights into disease mechanisms and treatment effects. Collaboration among researchers, clinicians and industry partners will be essential for translating promising preclinical findings into effective therapies for PAH patients.

Innovations in genetic engineering, such as CRISPR/Cas9, have also contributed to identifying molecular targets in PH, although they do not yet fully replicate the human disease. No genetically modified mouse

or rat should be considered a comprehensive model of PH. The underlying mechanisms in these models are often linked to deleterious or overexpressed genes, or alterations in the serotoninergic system (*e.g.* Fawn Hooded rats or serotonin transporter overexpressing mice). However, PAH is a chronic and multifactorial disease that cannot be reduced to a single genetic alteration or pathway.

Conclusion

While significant strides have been made in understanding and treating PAH, challenges persist in translating these advances into effective clinical therapies. The complexity and heterogeneity of PAH necessitate improved preclinical models and a deeper exploration of its cellular and molecular underpinnings. New technologies, such as single-cell sequencing, organ-on-chip models, and proteomic studies, could help personalise therapies and improve outcomes, particularly by identifying reliable and easily measurable biomarker panels for routine clinical use. To bridge the gap between preclinical research and patient care, a more integrated approach is required, combining robust preclinical data with human observations. Collaboration among researchers, clinicians and industry stakeholders is essential for accelerating the development of innovative therapies.

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