Strategic Plan for Lung Vascular Research

An NHLBI-ORDR Workshop Report

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The Division of Lung Diseases of the National Heart, Lung, and Blood Institute, with the Office of Rare Diseases Research, held a workshop to identify priority areas and strategic goals to enhance and accelerate research that will result in improved understanding of the lung vasculature, translational research needs, and ultimately the care of patients with pulmonary vascular diseases. Multidisciplinary experts with diverse experience in laboratory, translational, and clinical studies identified seven priority areas and discussed limitations in our current knowledge, technologies, and approaches. The focus for future research efforts include the following: (*1*) bet-

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Basic lung vascular research is progressing and novel translational and clinical study opportunities are emerging, particularly for pulmonary arterial hypertension. The investigative community is assessing how to move forward to acquire new knowledge, apply new technologies, and develop new tools to conduct modern studies in lung vasculature research so that lung health may be improved.

What This Study Adds to the Field

This report represents a collective body of scientific expert opinion provided to the National Heart, Lung, and Blood Institute for use in strategic support planning. The recommendations given here will be of interest to the general cardiopulmonary community because they constitute a summary of the directions lung vascular research may take in the near future.

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ter characterizing vascular genotype–phenotype relationships and incorporating systems biology approaches when appropriate; (*2*) advancing our understanding of pulmonary vascular metabolic regulatory signaling in health and disease; (*3*) expanding our knowledge of the biologic relationships between the lung circulation and circulating elements, systemic vascular function, and right heart function and disease; (*4*) improving translational research for identifying disease-modifying therapies for the pulmonary hypertensive diseases; (*5*) establishing an appropriate and effective platform for advancing translational findings into clinical studies testing; and (*6*) developing the specific technologies and tools that will be enabling for these goals, such as question-guided imaging techniques and lung vascular investigator training programs. Recommendations from this workshop will be used within the Lung Vascular Biology and Disease Extramural Research Program for planning and strategic implementation purposes.

Keywords: right ventricle; pulmonary hypertension; metabolism; genomics; phenotyping

Lung perfusion is accomplished by the pulmonary circulation, which originates from the right ventricle, and the bronchial circulation, which originates from the aorta. The low-resistance characteristics of the pulmonary circulation allow it to accommodate the entire cardiac output while maintaining low pulmonary vascular pressures, thereby preventing hydrostatic damage to the delicate alveolar blood–gas barrier. The bronchial circulation is approximately 3% of total lung perfusion and provides most of the nutrients and oxygen to the airways and the large pulmonary vessels via the vasa vasorum. In addition, the lung lymphatic vessels remove extravascular water and protein.

Despite significant discoveries in vascular biology, there remain gaps in our knowledge of lung diseases characterized by vascular remodeling, proliferative vessel growth, and/or loss of the pulmonary vascular bed. One lung vascular disease is pulmonary arterial hypertension (PAH), which is now described as a panvasculopathy of elastic, muscular, and nonmuscular pulmonary arteries and arterioles. Although a rare disorder (1), major improvements in the lives of patients with PAH have directly resulted from basic lung vascular research. However, without disease-modifying therapies, PAH remains a progressive and rapidly fatal disease. Furthermore, a report from the Centers for Disease Control and Prevention (Atlanta, GA) indicated that during 1980–2002, death rates and hospitalization rates significantly increased for "pulmonary hypertension" as either any contributing cause of death or as any listed hospital diagnosis (2). The etiology of this observation was hypothesized to be multifactorial, but the economic impact of this trend was clear, as was the need to further advance our scientific understanding of lung vascular health and disease, particularly in an aging U.S. population.

To expedite progress in lung vascular research, an invitational workshop of leading experts in laboratory, translational, and clinical studies was held. The objectives of the workshop were to review the state of science in lung vascular biology, identify emerging opportunities, define research directions, and make recommendations to the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH, Bethesda, MD) to use for strategic planning. Emphasis on PAH emerged, because several translational research opportunities were identified specific to this clinical condition. Although a primary focus on the pulmonary circulation is presented here, we acknowledge that key areas for investigation exist specific to the bronchial and lung lymphatic networks, but time constraints did not allow for open discussion of all topics. Selected slides presented at the meeting are included in the online supplement.

PRIORITY AREAS

Integrating "-omics" and Systems Biology Approaches

The study of pulmonary vascular disease has been accelerated by genomic discoveries, but the potential for even greater impact on disease pathogenesis and treatment may be attained with the advent of systems biology approaches. Much of what we are learning is being derived from studies of familial PAH. In 2000, researchers from Columbia University (New York, NY) and Vanderbilt University (Nashville, TN) reported that a mutation in a transforming growth factor (TGF)-β receptor superfamily member was associated with familial PAH (3, 4). Heterozygous germ line mutations of bone morphogenetic protein receptor-2 (BMPR2) underlie up to 80% of cases of familial PAH (3, 4). Gene rearrangements of BMPR2 are also common (5, 6). Investigation of other signaling molecules within the BMPR2 pathway has led to the discovery of other mutations associated with PAH. Patients with hereditary hemorrhagic telangiectasia may exhibit pulmonary hypertension. In these cases, coding changes in activin receptor–like kinase-1 (ALK1) are associated with disease (7). Kindred functional analysis of ALK1 shows that the mutations are the cause of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia (8). Endoglin, an accessory TGF-β receptor that is highly expressed during angiogenesis, is essential for ALK1 signaling. Mutations in endoglin have also been reported in patients with PAH (9). Thus, disruptions of the BMPR2 pathway are present in most cases of familial PAH (10). The impact of BMPR2 mutations in affecting global lung vascular function and disease will need to be ascertained by continued investigation.

In addition to better understanding sequence variations, it is becoming clear that epigenomic regulation plays an important role in the manifestation of lung vascular disease (11). Proliferation, migration, survival, and inflammation are processes critically regulated by protein posttranslational changes. The present understanding of posttranslational modifications affecting phenotypic responses during pulmonary vascular remodeling is limited. Histone modifications can induce epigenetic changes, affecting gene expression and phenotype long after the stimulus is removed. These changes are likely to play key roles in regulating phenotypic and epigenetic responses in pulmonary vascular diseases.

Advances in our understanding of genetics and epigenetics in lung vascular health and disease may be achieved by employing novel methodologies and analysis techniques. Expression studies (transcriptional profiling) on lung tissue are limited by small sample sizes (12). However, alternative strategies using surrogate tissue (peripheral blood) validate the utility of transcriptional profiling (13). Gleaning information from diseased lung tissue samples more easily obtained by biopsy may also provide useful information for lung vascular disease. For example, a sampling of lung tissue expression array analysis demonstrates similar pathway disruption among PAH and pulmonary fibrosis (14). Finally, putting all relevant "-omic" information into a systems biology model of pulmonary vascular disease may provide unique insights (15).

Research Opportunities

Determine DNA variation. Examination of DNA sequence variation related to disease states and defined phenotypes may enable highly accurate determination of the importance of rare variants.

Explore gene expression and control of transcription. A broad approach to analysis of gene expression, including involved tissues, laser capture of defined elements, cell line–based exam-

inations, and surrogate tissues such as blood, will likely be required. Technologies have emerged to examine the control of transcription including epigenomic modifications and the role of microRNA and RNA-binding proteins in disease processes.

Define epigenetic modifications. Epigenetic mechanisms alter gene expression responses in vascular and progenitor cells. Basic studies to define these mechanisms in the context of vascular remodeling will be important.

Identify protein posttranslational modifications. Modifications such as thiol-redox changes likely enhance pulmonary vascular disease. Distinguishing static and constitutive modifications from those responsible for the reversible regulation of pathogenic cell behavior is an important goal.

Define proteomic and metabolomic signatures. Augmented capacities in proteomic technologies enable broader examination of proteomic profiles, posttranslational modification, and metabolomic signatures. Application of these technologies holds promise for discovering disease pathogenesis and biomarker discovery/validation.

Institute systems biology approaches. Integration of broad-based approaches is essential for better defining the pathogenesis of lung vascular disease and therapeutic interventions. Network analysis can be derived for simple canonical system motifs, or more complex, scale-free, systems may be envisioned to examine the potential for disease similarities by common hubs and nodes. Application of computational biology is expected to reveal new diagnostic and therapeutic targets.

Identify specific clinical cohorts. A comprehensive and integrated approach to patient enrollment and development of databases of large cohorts is the best method to define phenotypes. Because lung vascular disease has protean manifestations, yet remains relatively rare, a consortium approach to acquisition of cohorts will likely be required.

Metabolism

Metabolic signaling contributes importantly to cellular behavior in pulmonary vascular disease. Improved understanding of the systems regulating these signals is important for identifying potential therapeutic approaches. Interactions among these systems likely create synergisms that align with environmental and genetic factors to initiate or promote lung vascular problems. In PAH, the observations of increased glycolysis (16) in the pulmonary vasculature coupled with a hypertrophied right ventricle indicate the need for comprehensive profiling of the metabolism of both heart and lung.

Hypoxia and shear stress, recognized triggers in the development of vascular remodeling, alter cellular metabolic signaling pathways that regulate proliferation, migration, cell survival, inflammation, and other components of pulmonary vascular diseases (17–22). Reactive oxygen species (ROS) mediate many of the cellular responses to hypoxia, shear stress, and TGF-β family members. ROS arise from multiple sources, including mitochondria, NAD(P)H oxidase complexes, and cytochrome P-450s. Genetic variation affecting estrogen metabolism, such as CYP1B1, a member of the cytochrome P-450 family, may underlie the enhanced susceptibility of women to PAH. Understanding the mechanisms responsible for regulating ROS production in various cell types, the targets of oxidant signals, and ROS contributions to pulmonary vascular disease is important for both pathogenesis and potential treatments.

Signaling pathways involving mitochondria, including fission and fusion, may influence vascular remodeling. Altered glucose utilization by lung vascular cells may enhance the proliferation potential, although our understanding of the underlying mechanisms is limited. Metabolic syndrome and insulin resistance alter mitogen-activated protein kinase–induced cell proliferation, decrease nitric oxide synthesis and adiponectin secretion, and increase inflammation through enhanced signaling via receptors for advanced glycation end products (RAGE). BMPR2 signaling promotes cell migration by a RAGE-dependent mechanism, and inhibits proliferation through peroxisome proliferator–activated receptor (PPAR)-γ signaling. Other metabolic signaling systems are implicated in pulmonary vascular disease, including L-arginine/polyamine metabolism, leptin signaling, and serine/threonine kinase pathways including the mammalian target of rapamycin (mTOR) and phosphoinositide 3-kinase/Akt.

Research Opportunities

Characterize oxidant signaling. In subcellular compartments of vascular, progenitor, and stem cells, and in the context of pulmonary vascular remodeling, elucidating oxidant signaling pathways and regulation is critical for defining mechanisms.

Understand cellular bioenergetics. Mitochondria regulate cellular bioenergetics, NAD(P)H redox, stress signaling, calcium, and ROS generation. Alterations in mitochondrial biosynthesis, autophagy, and fission/fusion affect cellular behavior. Changes in glucose utilization (e.g., the "Warburg effect") may contribute to pulmonary vascular cell proliferation.

Explore BMPR2 signaling and identify factors that synergize with BMPR2 mutations. The mechanisms by which BMP signals regulate cell proliferation remain elusive, and the contributions of PPAR-γ, RAGE, and their interactions with β-catenin to elicit lung vascular disease should be explored.

Advance basic understanding of estrogen metabolism, L-arginine/nitric oxide, polyamine signaling, mTOR, and cell–matrix signaling. These biologic processes have strong associations with enhancing the incidence or severity of lung vascular disease. The arena of cell–extracellular matrix interactions remains underdeveloped in pulmonary vascular pathobiology, and yet these features are fundamental in controlling cell behavior and vascular stiffness. Collaborations with experts in bioengineering who investigate the regulation of matrix assembly would enhance studies in this area.

Integrate metabolic syndrome research. Hyperinsulinemia, decreased adiponectin, altered leptin signaling, inflammation, and other manifestations of metabolic syndrome appear to accelerate lung vascular disease (22, 23). Research enhancing understanding of the relationships between global metabolic dysfunction and lung circulatory disease should be continued.

Circulating Elements

Endothelial cells lining the pulmonary vasculature display great heterogeneity with respect to cell surface antigen expression, metabolic and physiologic activity, barrier properties, morphology, and proliferative potential (24–27). Circulating red blood cells can modulate NO signaling in the lung vasculature, by viscosity and shear-mediated mechanotransduction of endothelial NO synthase activation, by hemoglobin-dependent NO scavenging, and by production of vasodilatory mediators such as nitrite and ATP. Red cell hemolysis releases vasoconstrictive factors such as hemoglobin and arginase-1 that produce endothelial dysfunction (28). It is increasingly apparent that circulating "endocrine" vasodilatory mediators in blood such as nitrite, derived from the diet or endothelial NO synthase, can be delivered via the bloodstream and converted to NO to modulate pulmonary endothelial responses to hypoxia (29, 30). Likewise, pulmonary endothelial cells release metabolic and endocrine factors that stimulate the growth and differentiation

of epithelial cells (31), demonstrating that the lung endothelium is integrating information locally and from the systemic circulation to alter lung function.

Cell-derived vesicles are heterogeneous particles containing cell membrane proteins, metabolites, DNA, and RNA (including mRNA and microRNA) from numerous cell types within the circulation (32). Release of vesicles into the circulation occurs on cell stress or injury or in response to a host of diseases (33, 34). Vascular endothelium responds to circulating vesicles by changing its gene expression profile and function (35). Circulating vesicles may play roles in tissue and cell repair and regeneration or may contribute to organ damage.

The relationship between resident lung stem cells and circulating cells, and the paracrine signaling effects they produce to participate in vascular repair and lung regeneration, remain unknown. Circulating proangiogenic cells that are derived from the hematopoietic system have also been identified and used as biomarkers of lung injury; however, only resident or the rare circulating endothelial cells are able to form vessels *in vivo* (36, 37). Pulmonary vascular endothelium possesses proliferative potential (38), but endothelial stem cells capable of repopulating vessels within the lung have been recognized only more recently. There is a need to improve our understanding of the functional heterogeneity of vascular cells in different segments of the lung vascular beds, using high-throughput approaches, including large-scale proteomics and phage display (39). The systemic bronchial vascular endothelium may have greater proliferative capacity than the pulmonary vascular endothelium, but factors influencing this are unknown. Mice possess lung cells that can be isolated and enriched for lung vascular repopulating cells in older, lethally irradiated congenic recipients. Rare, stemlike cells engraft and proliferate as colonies within the recipient vasculature. Once lung stem cell–derived vessels are functional they appear to enhance the engraftment of transplanted pulmonary epithelial cells.

Understanding dynamic remodeling (i.e., growth and involution) of the lung circulation impacts on regenerative therapies aimed not only at pulmonary vascular diseases, but other lung diseases, including interstitial lung diseases (40) and emphysema (41). Another area of emerging understanding includes the critical contributions of the immune system in terms of immune surveillance of the vascular milieu.

Research Opportunities

Explore lung vascular regeneration potential. Opportunities exist to define whether the lung harbors cells that repair or regenerate endothelium and to determine where they reside, how they can be isolated, what homing molecules they express, and whether there are specific niches into which they engraft. Studying how circulating bone marrow–derived proangiogenic cells amplify or remodel the lung vasculature should be a research priority.

Advance cell-derived vesicle research. There is a need to develop tools that specifically identify, quantify, and analyze the molecular composition of circulating cell–derived vesicles and to correlate their number and function with lung vascular health and disease in animal models of human cardiopulmonary diseases and human subjects.

Construct model systems. There is a lack of model systems for understanding the function of the pulmonary endothelium as a dynamic system, which integrates circulating cellular and molecular input from all types of circulating elements (e.g., normal and diseased erythrocytes, erythrocyte products, platelets and platelet products, leukocytes and leukocyte products, plasma proteins).

Vascular Cross-talk between Pulmonary and Systemic Circulations

Whereas the uniqueness of the pulmonary circulation proper rests with its hypoxic vasoconstriction physiological response, we know little of lung vasculature involvement in integrating overall vascular health and disease development. The complexity of overall lung circulatory biology is enhanced because of the potential for differential physiological and pathophysiological roles of lung vascular components, that is, pulmonary veins, bronchial circulation, and lung lymphatics. Concepts derived from studies in the systemic circulation have highlighted several potential areas of interest in the overall lung circulation. The use of angiogenesis inhibitors, particularly of vascular endothelial growth factor (VEGF) signaling, has underscored the dynamic nature of vascular regression and regrowth; in fact, the basement membrane left behind by the regressing vasculature provides "tracks" for the regrowth of blood vessels (42). The tumor environment is conducive to vascular regeneration as it is rich in proangiogenic factors (43), but antiangiogenic approaches collaterally damage circulatory beds that rely on growth factor signaling, such as the lung.

Systemic vascular beds and the pulmonary circulation do interact, perhaps best exemplified by pulmonary hypertension due to liver cirrhosis and hepatopulmonary syndrome (44). Some form of pulmonary vasodilation is present in 60% of cirrhotic patients, with 30% having gas exchange abnormalities. Pulmonary hypertension is seen in about 6% of all cirrhotic patients. Investigations into the pathogenesis of experimental hepatopulmonary syndrome based on bile duct ligation have uncovered an increased interaction of circulating monocytes and the pulmonary circulation, leading to the production of mediators such as nitric oxide and carbon monoxide (45). These alterations are accompanied by increased alveolar capillary density and correlate with increased expression of VEGF and VEGF receptor-2. Furthermore, renal–lung interactions have been found in models of acute renal injury, leading to permeability changes in the lung (46).

Advances in live optical imaging, when combined with genetic interventions, provide a powerful approach for studying key signaling events in the systemic and pulmonary circulation (47). These approaches have the added advantage of allowing for cellular responses in the multicellular context of the intact lung. For example, real-time assessment of neutrophil migration through capillaries can be visualized concomitantly with cellular signaling events, including calcium fluxes and ROS generation. The incorporation of these tools for research and eventually for diagnosis, which are well developed in studies of the systemic circulation and tumor vascular biology, is critical for the future studies in lung circulation.

Research Opportunities

Develop imaging technology for the pulmonary circulation. Developing live molecular and structural imaging will offer insight into novel molecular markers and molecular pathways involved in the control of the pulmonary (arterial and venous), bronchial, and lymphatic circulations. Visualizing real-time cell–cell communications in the lung will be possible with nanotechnology, as will detecting altered metabolic signaling. Imaging efforts need a strong training component in multidisciplinary approaches that leverage expertise in chemistry, physics, experimentation, and clinical problems, among others. Fostering programs between academia and industry would be desirable, as would enhancing access to shared imaging centers. The creation of core facilities run by imaging scientists and equipped with high

field strength magnetic resonance imaging (MRI), micro-PET/SPECT/CT (positron emission tomography/single-photon emission computed tomography/computed tomography), high-frequency Doppler imaging, and advanced optical imaging should be encouraged at national centers of excellence in cardiopulmonary research. Improving imaging will lead to improved early disease detection.

Explore the relationships between systemic vasculature and pulmonary vascular function. Abnormalities in systemic vascular reactivity and function that occur in relation to pulmonary vascular disease may reveal a new understanding of pulmonary vascular pathophysiology. There is a need to understand why a genetic or environmental insult results in vascular disease in the lung, rather than in other organs.

The Lung Vascular–Cardiac Axis

It has become apparent that the pulmonary circulation is intimately coupled to right ventricular health and disease. Severe forms of PAH (including idiopathic forms) continue to be treated with a single vasodilator agent or a combination of vasodilator drugs (48). After more than a decade of clinical experience with this approach it is clear that in too many instances a significant and lasting reduction of the right ventricular afterload cannot be achieved, and patients with PAH die of right heart failure (49). Prevention of the development of right ventricular failure (RVF) independent of attempts to reduce the RV afterload has not been a treatment goal. There is lack of robust and validated diagnostic criteria that describe early phases of RV dysfunction (50) No detailed knowledge base exists regarding the transition from RV hypertrophy (RVH) to RVF in the setting of the remodeled lung circulation in PAH (51). However, acquisition of this knowledge is critical given the observation that RV function can fully recover within weeks of lung transplantation in patients with end-stage PAH. How the failing RV returns to normal function after lung transplantation is unknown.

Our concepts of RVF mechanisms have been shaped largely by investigations of left ventricular failure, even though there is evidence that the right and left ventricles differ in responses to increased afterload. For example, right ventricular systolic pressure undergoes a four- to fivefold increase above normal during the development of severe pulmonary hypertension whereas the left ventricular systolic pressure in the setting of aortic stenosis undergoes a small percent change only. In addition, it is known that α_1-adrenergic agonists increase the contractile force of the left ventricle, whereas they cause a force reduction in the corresponding normal RV (52), and long-term infusion of norepinephrine leads to left ventricle hypertrophy whereas the RV does not undergo hypertrophy (53). Other differences between right and left ventricular biology are being discovered, including developmental programs and resident stem cell populations.

Research Opportunities

Validate diagnostic criteria of RV dysfunction. Candidate noninvasive (echocardiographic) variables include tricuspid valve annular plane systolic excursion, RV fractional shortening (54, 55), and isovolumetric acceleration. Cardiac MRI has become the reference standard modality for evaluation of cardiac anatomy, function, and remodeling. New imaging markers for afterload need to be further explored, including main pulmonary artery mean flow using phase-contrast MRI. The role of myocardial perfusion reserve in RV dysfunction is unknown and should be explored. Candidate MRI variables include RV volumes, RV wall strain, and RV perfusion. Three-dimensional echocardiography of the RV could substitute for MRI when device contraindications exist or could complement MRI.

Improve understanding of RVH and RVF development. Key areas to address include the following: the role of the adrenergic receptor system in RVF; an understanding of whether RVH in PAH is an adaptive compensatory mechanism similar to left ventricular hypertrophy in the athlete; identification of the processes that lead to adaptive and "functional" RVH; identification of mechanisms of the transition from RVH to RVF; advancements in understanding the metabolic changes characteristic of RVH and RVF; investigating whether RVF is a form of myocardial hibernation; and the role of phosphodiesterase inhibitors in RVH and/or RVF.

Develop imaging techniques for assessing RV remodeling. A switch to glycolysis (from fatty acid oxidation) reflects cardiac hypertrophy and indicates hyperpolarized mitochondria in RV remodeling. Glucose uptake as indicated by the uptake value of fluorodeoxyglucose by PET may correlate with vascular remodeling and RV function in PAH. The identification of RV dysfunction biomarkers and/or strain would complement imaging studies.

Discover and design directed therapies to prevent (and reverse) RVF. Model animal studies are needed to explore cellular and molecular mechanisms of RVF (56, 57). Potential targets for study include neurohormonal activation and mechanisms for preventing RV capillary loss, restoring blood flow, and decreasing fibrosis to improve energy utilization. Trials need end points reflective of the treatment of RV dysfunction and failure. Assessment of the heart directly by endomyocardial biopsy (genomic, proteomic analysis) must also be considered.

Advance collective organization of research efforts. A conceptual approach that integrates cardiac and pulmonary vascular evaluations should be advanced, including left ventricular function knowledge. Types of studies might include evaluation of the transcriptomes and proteomes from the right and left ventricles and determination of how the left ventricle is affected by ventricular interdependence in the setting of PAH and RVF.

Discovery of Novel PAH Treatments

Although a minority (<15%) of patients with severe PAH respond significantly to acute pulmonary vasodilators and can be treated successfully with calcium channel blockers, most are not responsive to vasodilators and are currently treated with prostacyclin analogs, endothelin-1 receptor blockers, type 5 phosphodiesterase inhibitors, or various combinations of these agents (58). These treatments provide some improvement in the quality of life of patients, but there is no convincing evidence that they significantly prolong survival (59). Overall, despite the use of many expensive drugs, PAH remains a debilitating and deadly disease and more effective therapies are urgently needed. The pathogenesis of PAH is generally ascribed to vasoconstriction, vascular wall remodeling, and *in situ* thrombosis. It is also generally believed that whereas vasoconstriction may be important in the early stages, the major factor responsible for the high pulmonary vascular resistance in severe, established PAH is the formation of occlusive neointimal and plexiform lesions in small, peripheral pulmonary arteries. It is likely that these hypercellular and/or fibrotic lesions will have to be "dissolved" or bypassed by new vessel growth to effectively reverse the high resistance and pressure of PAH. This presents a formidable challenge, because we do not yet understand exactly what causes the formation of these vascular lesions or the cellular and molecular mechanisms involved.

Research Opportunities

Advance pharmacogenomic approaches. Pharmacogenomic approaches (60) are now feasible to identify the roles of gene polymorphisms in determining the differences among patients with PAH and the predicted efficacy and/or toxicity of therapy. This includes considering both vascular-directed and right ventricular–directed treatments, thereby offering personalized therapy for PAH based on identification of individuals likely to receive the most benefit at least risk.

Improve drug delivery. Homing peptides (61) to the pulmonary vascular bed should be evaluated to optimize the selective delivery of drugs to specific vascular segments and cells.

Develop methods to identify regression of vascular lesions. Tracking both the formation and dissolution of occlusive pulmonary vascular lesions may be accomplished via high-resolution angiographic or nuclear imaging using radioisotope-labeled particulates or biologics that specifically target vessel legions.

Improve animal models of pulmonary vascular disease. Animal models that closely mimic the hemodynamic pathophysiology and occlusive neointimal and plexiform pulmonary arteriopathy of human PAH will be essential for more rigorous preclinical testing of new therapies (62).

Improving PAH Care through Human Subject Studies

Clinical research efforts in lung vascular disease have focused on PAH. The management of PAH has advanced since the publication of the NIH-supported 1980s registry, which established a prognostic benchmark for survival that is still in use today (63, 64). The French Network on Pulmonary Hypertension has investigated contemporary survival during a 3-year study of adult patients with idiopathic, familial, or anorexigen-associated PAH. Using the NIH model, survival in incident cases has improved by only about 10–15%. Higher mortality was closely associated with male sex, right ventricular function, and exercise limitation. Nevertheless, the long-term management of patients with PAH beyond the initiation of drug therapy is poorly studied and understood (65, 66). A prospective study of epidemiological risk for PAH has not been performed. Such studies would require a large cohort for informative analysis. Understanding the mechanisms of human pulmonary vascular disease will require efforts similar to those made in more common cardiovascular diseases, that is, large, multicenter studies. Indeed, pulmonary hypertension studies should be considered as a component of national studies such as MESA (Multi-Ethnic Study of Atherosclerosis) (67) whenever possible. Most simple and important questions, such as the value of anticoagulation or of introducing aspirin or β blockade, have not been systematically addressed.

The design of future pulmonary hypertension clinical studies must include improved clinical measurements, the marriage of basic scientific aims to clinical trials (68), the development of surrogate survival end points, the development of large databases, standardized methods of precise phenotyping across centers (including molecular phenotyping), protocolized methods for sample collection and storage, and complete data collection with open access (69, 70).

Research Opportunities

Identify new clinical studies in PAH. The efficacy of long-term therapy should be addressed, including the systematic evaluation of therapies bearing low risk and low cost that show substantial impact in systemic vascular diseases (e.g., warfarin, exercise, and antiplatelet therapy). Routine pulmonary arterial "stiffness" measurements are possible for assessing the pulmonary circulation (71–74) and validated measures of vascular stiffness and ventricular–vascular coupling should be incorporated into clinical trials.

Implement an appropriate research support structure. A consortium of research centers should serve to form a modern translational and clinical research support platform. A key function of a consortium would be to perform phenotyping. Associations with pharmaceutical clinical trials could be leveraged for simultaneous cost-effective collection of data and biobanked materials for use in common research. Basic questions of risk and etiology in PAH must be addressed by a multicenter, prospective study of a large number of carefully phenotyped patients.

Enhance training of lung vascular investigators. Training components supported by a consortium or other mechanisms are necessary to ensure that the next generation of lung vascular scientists is equipped to move forward.

Summary of Recommendations to the Institute

To be used for planning and prioritization in concert with the NHLBI mission, formal recommendations from this workshop are summarized as follows:

- Advance basic scientific research in lung vascular biology, utilizing emerging technologies.
- Advance and coordinate basic and clinical knowledge of the pulmonary circulation–right heart axis through novel research efforts, utilizing multidisciplinary teams.
- Define interactions between lung vascular components and circulating elements and systemic circulations by fostering novel collaborations.
- Encourage systems biology analysis to understand and define interactions between lung vascular genetics, epigenetics, metabolic pathways, and molecular signaling.
- Develop strategies using appropriate animal models to improve the understanding of the lung vasculature in health and in conditions that reflect human disease.
- Enhance translational research in lung vascular disease by comparing cellular and tissue abnormalities identified in animal models with those in human specimens.
- Improve lung vascular disease molecular and clinical phenotype coupling.
- Develop *in vivo* imaging techniques that assess structural changes in lung vasculature, metabolic shifts, functional cell responses, and right ventricular function.
- Develop research consortia that advance basic, translational, and clinical studies; allow for multicenter epidemiological study feasibility; and support training of junior investigators in lung vascular biology and disease.

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References

1. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, *et al.* Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* 2010;122:156–163.
2. Hyduk A, Croft JB, Ayala C, Zheng K, Zheng ZJ, Mensah GA. Pulmonary hypertension surveillance—United States 1980–2002. *MMWR Surveill Summ* 2005;54:1–28.
3. International PPH Consortium; Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA III, Loyd JE, Nichols WC, Trembath RC. Heterozygous germline mutations in *BMPR2*, encoding a TGF-β receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000;26:81–84.
4. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, *et al.* Familial primary pulmonary hypertension (gene *PPH1*) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000;67:737–744.
5. Aldred MA, Vijayakrishnan J, James V, Soubrier F, Gomez-Sanchez MA, Martensson G, Galie N, Manes A, Corris P, Simonneau G, *et al.* *BMPR2* gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat* 2006;27:212–213.
6. Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, Stanton KC, Wheeler LA, Phillips JA III, Loyd JE, *et al.* High frequency of *BMPR2* exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2006; 174:590–598.
7. Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Galie N, Loyd JE, Humbert M, *et al.* Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001;345:325–334.
8. Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, Elliott CG, Robbins IM, Olschewski H, McLaughlin V, *et al.* Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet* 2003;40:865–871.
9. Chaouat A, Coulet F, Favre C, Simonneau G, Weitzenblum E, Soubrier F, Humbert M. Endoglin germline mutation in a patient with hereditary haemorrhagic telangiectasia and dexfenfluramine associated pulmonary arterial hypertension. *Thorax* 2004;59:446–448.
10. Machado RD, Eickelberg O, Elliott CG, Geraci MW, Hanaoka M, Loyd JE, Newman JH, Phillips JA III, Soubrier F, Trembath RC, *et al.* Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol* 2009;54:S32–S42.
11. Archer SL, Marsboom G, Kim GH, Zhang HJ, Toth PT, Svensson EC, Dyck JR, Gomberg-Maitland M, Thebaud B, Husain AN, *et al.* Epigenetic attenuation of mitochondrial superoxide dismutase 2 in pulmonary arterial hypertension: a basis for excessive cell proliferation and new therapeutic target. *Circulation* 2010;121:2661–2671.
12. Geraci MW, Moore M, Gesell T, Yeager ME, Alger L, Golpon H, Gao B, Loyd JE, Tuder RM, Voelkel NF. Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis. *Circ Res* 2001;88:555–562.
13. Bull TM, Coldren CD, Moore M, Sotto-Santiago SM, Pham DV, Nana-Sinkam SP, Voelkel NF, Geraci MW. Gene microarray analysis of peripheral blood cells in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2004;170:911–919.
14. Rajkumar R, Konishi K, Richards T, Ishizawar D, Wiechert A, Kaminski N, Ahmad F. Genome-wide RNA expression profiling in lung identifies distinct signatures in idiopathic pulmonary arterial hypertension and secondary pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2010;298:H1235–H1248.

15. Loscalzo J, Kohane I, Barabasi AL. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. *Mol Syst Biol* 2007;3:124.
16. Xu W, Koeck T, Lara AR, Neumann D, DiFilippo FP, Koo M, Janocha AJ, Masri FA, Arroliga AC, Jennings C, *et al.* Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci USA* 2007;104:1342–1347.
17. Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1α-Kv1.5 O_2-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol* 2008;294:H570–H578.
18. Hansmann G, Rabinovitch M. The protective role of adiponectin in pulmonary vascular disease. *Am J Physiol Lung Cell Mol Physiol* 2010;298:L1–L2.
19. Hassoun PM, Mouthon L, Barbera JA, Eddahibi S, Flores SC, Grimminger F, Jones PL, Maitland ML, Michelakis ED, Morrell NW, *et al.* Inflammation, growth factors, and pulmonary vascular remodeling. *J Am Coll Cardiol* 2009;54:S10–S19.
20. Ward JP, McMurtry IF. Mechanisms of hypoxic pulmonary vasoconstriction and their roles in pulmonary hypertension: new findings for an old problem. *Curr Opin Pharmacol* 2009;9:287–296.
21. Waypa GB, Marks JD, Guzy R, Mungai PT, Schriewer J, Dokic D, Schumacker PT. Hypoxia triggers subcellular compartmental redox signaling in vascular smooth muscle cells. *Circ Res* 2009;106:526–535.
22. Zamanian RT, Hansmann G, Snook S, Lilienfeld D, Rappaport KM, Reaven GM, Rabinovitch M, Doyle RL. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J* 2009;33:318–324.
23. Robbins IM, Newman JH, Johnson RF, Hemnes AR, Fremont RD, Piana RN, Zhao DX, Byrne DW. Association of the metabolic syndrome with pulmonary venous hypertension. *Chest* 2009;136:31–36.
24. Aird W. Phenotypic heterogeneity of the endothelium. I. Structure, function, and mechanisms. *Circ Res* 2007;100:158–173.
25. Gebb S, Stevens T. On lung endothelial cell heterogeneity. *Microvasc Res* 2004;68:1–12.
26. Ofori-Acquah SF, King J, Voelkel N, Schaphorst KL, Stevens T. Heterogeneity of barrier function in the lung reflects diversity in endothelial cell junctions. *Microvasc Res* 2008;75:391–402.
27. Stevens T, Phan S, Frid MG, Alvarez D, Herzog E, Stenmark KR. Lung vascular cell heterogeneity: endothelium, smooth muscle, and fibroblasts. *Proc Am Thorac Soc* 2008;5:783–791.
28. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. *Curr Opin Hematol* 2009;16:515–523.
29. Lundberg JO, Gladwin MT, Ahluwalia A, Benjamin N, Bryan NS, Butler A, Cabrales P, Fago A, Feelisch M, Ford PC, *et al.* Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol* 2009;5:865–869.
30. Zuckerbraun BS, Shiva S, Ifedigbo E, Matthier MA, Mollen KP, Rao J, Bauer PM, Choi JJ, Curtis E, Choi AM, *et al.* Nitrite potently inhibits hypoxic and inflammatory pulmonary arterial hypertension and smooth muscle proliferation via xanthine oxidoreductase-dependent nitric oxide generation. *Circulation* 2010;121:98–109.
31. Yamamoto H, Yun EJ, Gerber HP, Ferrara N, Whitsett JA, Vu TH. Epithelial–vascular cross talk mediated by VEGF-A and HGF signaling directs primary septae formation during distal lung morphogenesis. *Dev Biol* 2007;308:44–53.
32. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. *Trends Cell Biol* 2009;19:43–51.
33. Quesenberry PJ, Aliotta JM. The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. *Stem Cell Rev* 2008;4:137–147.
34. Zimmerman GA. Thinking small, but with big league consequences: procoagulant microparticles in the alveolar space. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1033–L1034.
35. Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc Res* 2005;67:30–38.
36. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2008;28:1584–1595.
37. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;109:1801–1809.
38. Alvarez DF, Huang L, King JA, El Zarrad MK, Yoder MC, Stevens T. Lung microvascular endothelium is enriched with progenitor cells that exhibit vasculogenic capacity. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L419–L430.
39. Giordano RJ, Edwards JK, Tuder RM, Arap W, Pasqualini R. Combinatorial ligand-directed lung targeting. *Proc Am Thorac Soc* 2009;6:411–415.
40. Farkas L, Farkas D, Ask K, Moller A, Gauldie J, Margetts P, Inman M, Kolb M. VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. *J Clin Invest* 2009;119:1298–1311.
41. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman SH, Hirth P, Waltenberger J, Voelkel NF. Inhibition of vascular endothelial growth factor receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000;106:1311–1319.
42. McDonald DM. Angiogenesis and vascular remodeling in inflammation and cancer. In: Figg WD, Folkman J, editors. Angiogenesis: an integrative approach from science to medicine, 1st ed. New York, NY: Springer; 2008. pp. 17–33.
43. Mehrad B, Keane MP, Strieter RM. Chemokines as mediators of angiogenesis. *Thromb Haemost* 2007;97:755–762.
44. Rodriguez-Roisin R, Krowka MJ. Hepatopulmonary syndrome—a liver-induced lung vascular disorder. *N Engl J Med* 2008;358:2378–2387.
45. Zhang J, Luo B, Tang L, Wang Y, Stockard CR, Kadish I, Van GT, Grizzle WE, Ponnazhagan S, Fallon MB. Pulmonary angiogenesis in a rat model of hepatopulmonary syndrome. *Gastroenterology* 2009;136:1070–1080.
46. Hassoun HT, Grigoryev DN, Lie ML, Liu M, Cheadle C, Tuder RM, Rabb H. Ischemic acute lung injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. *Am J Physiol Renal Physiol* 2007;293:F30–F40.
47. Kuebler WM, Parthasarathi K, Lindert J, Bhattacharya J. Real-time lung microscopy. *J Appl Physiol* 2007;102:1255–1264.
48. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med* 2004;351:1425–1436.
49. Bogaard HJ, Abe K, Vonk NA, Voelkel NF. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest* 2009;135:794–804.
50. Voelkel NF, Quaife RA, Leinwand LA, Barst RJ, McGoon MD, Meldrum DR, Dupuis J, Long CS, Rubin LJ, Smart FW, *et al.* Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. *Circulation* 2006;114:1883–1891.
51. Hein S, Arnon E, Kostin S, Schönburg M, Elsässer A, Polyakova V, Bauer EP, Klövekorn WP, Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 2003;107:984–991.
52. Wang G-Y, McCloskey DT, Turcato S, Swigart PM, Simpson PC, Baker AJ. Contrasting inotropic responses to α_1-adrenergic receptor stimulation in left versus right ventricular myocardium. *Am J Physiol Heart Circ Physiol* 2006;291:H2012–H2017.
53. Irlbeck M, Muhling O, Iwai Z, Zimmer HG. Different response of the rat left and right heart to norepinephrine. *Cardiovasc Res* 1996;31:157–162.
54. Giusca S, Dambrauskaite V, Scheurwegs C, D'Hooge J, Claus P, Herbots L, Magro M, Rademakers F, Meyns B, Delcroix M, *et al.* Deformation imaging describes RV function better than longitudinal displacement of the tricuspid ring (TAPSE). *Heart* 2010;96:281–288.
55. Innelli P, Esposito R, Olibet M, Nistri S, Galderisi M. The impact of ageing on right ventricular longitudinal function in healthy subjects: a pulsed tissue Doppler study. *Eur J Echocardiogr* 2009;10:491–498.
56. Bogaard HJ, Natarajan R, Henderson SC, Long CS, Kraskauskas D, Smithson L, Ockaili R, McCord JM, Voelkel NF. Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. *Circulation* 2009;120:1951–1960.
57. Bogaard HJ, Natarajan R, Kraskauskas D, Kraskauskiene V, Voelkel NF. β-Adrenergic blockade with carvedilol mitigates maladaptive right ventricular remodeling in rats with experimentally-induced pulmonary hypertension [abstract]. *Am J Respir Crit Care Med* 2009;179:A5120.
58. Rich S. The effects of vasodilators in pulmonary hypertension: pulmonary vascular or peripheral vascular? *Circ Heart Fail* 2009;2:145–150.
59. Macchia A, Marchioli R, Tognoni G, Scarano M, Marfisi R, Tavazzi L, Rich S. Systematic review of trials using vasodilators in pulmonary arterial hypertension: why a new approach is needed. *Am Heart J* 2010;159:245–257.
60. Pereira NL, Weinshilboum RM. Cardiovascular pharmacogenomics and individualized drug therapy. *Nat Rev Cardiol* 2009;6:632–638.

61. Ruoslahti E, Bhatia SN, Sailor MJ. Targeting of drugs and nanoparticles to tumors. *J Cell Biol* 2010;188:759–768.
62. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1013–L1032.
63. Rich S, Danzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK, *et al.* Primary pulmonary hypertension: a national prospective study. *Ann Intern Med* 1987;107:216–223.
64. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, *et al.* Survival in patients with primary pulmonary hypertension. *Ann Intern Med* 1991;115:343–349.
65. Minai OA, Nathan SD, Hill NS, Badesch DB, Stoller JK. Pulmonary hypertension in lung diseases: survey of beliefs and practice patterns. *Respir Med* 2010;104:741–748.
66. Ghofrani HA, Wilkins MW, Rich S. Uncertainties in the diagnosis and treatment of pulmonary arterial hypertension. *Circulation* 2008;118: 1195–1201.
67. Barr RG, Bluemke DA, Ahmed FS, Carr JJ, Enright PL, Hoffman EA, Jiang R, Kawut SM, Kronmal RA, Lima JA, *et al.* Percent emphysema, airflow obstruction, and impaired left ventricular filling. *N Engl J Med* 2010;362:217–227.
68. Archer SL, Weir EK, Wilkins MR. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. *Circulation* 2010;121:2045–2066.
69. Ventetuoio CE, Benza RL, Peacock AJ, Zamanian RT, Badesch DB, Kawut SM. Surrogate and combined end points in pulmonary arterial hypertension. *Proc Am Thorac Soc* 2008;5:617–622.
70. McLaughlin VV, Badesch DB, Delcroix M, Fleming TR, Gaine SP, Galiè N, Gibbs JS, Kim NH, Oudiz RJ, Peacock A, *et al.* End points and clinical trial design in pulmonary arterial hypertension. *J Am Coll Cardiol* 2009;54:S97–S107.
71. Champion HC, Michelakis ED, Hassoun PM. Comprehensive invasive and noninvasive approach to the right ventricle–pulmonary circulation unit: state of the art and clinical and research implication. *Circulation* 2009;120:992–1007.
72. Kawut SM, Al-Naamani N, Agerstrand C, Rosenzweig EB, Rowan C, Barst RJ, Bergmann S, Horn EM. Determinants of right ventricular ejection fraction in pulmonary arterial hypertension. *Chest* 2009;135: 752–759.
73. Gan CT-J, Lankhaar JW, Weterhof N, Marcus JT, Becker A, Twisk JWR, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Noninvasively assessed pulmonary artery stiffness predicts mortality in pulmonary arterial hypertension. *Chest* 2007;32:1906–1914.
74. Milnor WR, Conti CR, Lewis KB. Pulmonary arterial pulse wave velocity and impedance in man. *Circ Res* 1969;25:637–649.