The ENTELLIGENCE™ Young Investigator Program is supported through an educational grant from Actelion Pharmaceuticals US, Inc.
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Dear Colleagues,

We are delighted to announce that in 2016, the ENTELLIGENCE™ Young Investigator Program chose five new young investigators to receive ENTELLIGENCE awards based on their outstanding pulmonary vascular disease-related research proposals. These awards provide support to individual young investigators at universities and research institutes in the US and Canada to conduct basic science, translational, and/or clinical research through a 12-month mentored grant. For the past 11 years, members of the independent Steering Committee have selected cutting-edge projects that are scientifically relevant, original, and applicable to the problem of pulmonary vascular disease. More than 50 promising researchers in the field of pulmonary vascular disease have been awarded to date. In 2016, we received a record number of LOIs and grant applications.

Funded studies have targeted pulmonary vascular disease in the areas of pathophysiology, pharmacology, treatment, genetics, diagnosis, and epidemiology. Results from many of these projects have been presented at numerous key scientific meetings such as the American Thoracic Society, the American Heart Association, and the European Respiratory Society, and have been published in more than 40 peer-reviewed journals, including Nature, American Journal of Physiology, American Journal of Respiratory and Critical Care Medicine, Chest, Circulation, and Pulmonary Circulation. ENTELLIGENCE awardees have also advanced their careers in pulmonary vascular disease, with many becoming Assistant and Associate Professors of Medicine, Directors, Section Leaders, and mentors for up-and-coming young investigators.

The ENTELLIGENCE Young Investigator Program will soon begin another cycle of competition, continuing its commitment to advancing the understanding of pulmonary vascular disease and promoting the career development of young investigators planning an academic career in pulmonary vascular disease research. Please see important dates shown in the timeline below, visit the ENTELLIGENCE website and follow ENTELLIGENCE on social media:

On behalf of the ENTELLIGENCE Steering Committee, I would like to express our appreciation to Actelion Pharmaceuticals US, Inc. for making the ENTELLIGENCE Program possible and for graciously awarding a 5th grant this year.

Best regards,

Ronald J. Oudiz, MD
Program Overview

The ENTELLIGENCE Young Investigator Program
Supporting young investigators

The ENTELLIGENCE Young Investigator Program, established in 2005, provides opportunities for promising young investigators to promote quality medical care and enhance patients’ lives by supporting research (basic science, clinical, or translational) in the area of pulmonary hypertension (PH), specifically related to expanding knowledge of pulmonary vascular pathobiology pathways. The ENTELLIGENCE program is led by a Steering Committee comprised of leaders in the field of PH who award 12-month mentored grants of up to $100,000 to conduct basic science and clinical research. Grants are based on scientific merit, originality, feasibility, and applicability to the diagnosis and treatment of PH, conditional upon supervision by an appropriate mentor, and conducted at a university or research institute in the US or Canada. The program is consistent with Actelion’s commitment to basic science and clinical research in the area of PH.

The ENTELLIGENCE program is funded by an independent grant from Actelion Pharmaceuticals US, Inc. All decisions to fund protocols are solely decided by the ENTELLIGENCE Steering Committee and the receipt of a grant in no way requires the recipient, nor implies that the recipient is obligated to, recommend or prescribe any Actelion product.

How to submit

Applicants are invited to submit original basic or clinical investigations specifically targeting pulmonary vascular disease in the following areas: Pathophysiology, Pharmacology, Treatment, Genetics, Diagnosis, and Epidemiology. Applications are submitted electronically as a Letter of Intent. Submitted applications are reviewed by the Steering Committee and selected applicants are invited to submit full proposals. The timelines, submission procedure, and submission forms are available on the ENTELLIGENCE website www.entelligencemd.org.

Review cycles completed: 11
Awards distributed: 55
Overview of ENTELLIGENCE Awards

AWARDED 2016

Olivier Boucherat, PhD
Québec Heart and Lung Institute Research Centre
Québec, QC, Canada
Mentor: Sébastien Bonnet, PhD
Project Title: Impact of mitochondrial heat shock protein 90 inhibition in pulmonary arterial hypertension

Vitaly O. Kheyfets, PhD
University of Colorado Denver
Denver, CO
Co-investigator: Shelley Miyamoto, MD
Mentors: Robin Shandas, PhD and Dunbar Ivy, MD
Project Title: Inter-ventricular decoupling is an overlooked contributor to right ventricular myocardial stress and dysfunction in pediatric pulmonary hypertension

Todd M. Kolb, MD, PhD
Johns Hopkins University
Baltimore, MD
Co-investigator: David Kass, MD
Mentor: Paul Hassoun, MD
Project Title: PDE9A in right ventricular and pulmonary vascular remodeling

Jeffrey C. Robinson, MD
University of Colorado
Aurora, CO
Mentor: Rubin Tuder, MD
Project Title: Iron deficiency and hypoxic signaling in pulmonary hypertension

Haiyang Tang, PhD
University of Arizona
Tucson, AZ
Co-Investigator and Mentor: Jason Yuan, MD, PhD
Project Title: Differential role of mTORC1 and mTORC2 in hypoxic vasoconstriction and the development of pulmonary hypertension
Overview of ENTELLIGENCE Awards

Awarded 2015

Ketul R. Chaudhary, PhD
Ottawa Hospital Research Institute
Ottawa, ON, Canada
Mentor: Duncan J. Stewart, MD
Project Title: Genetic and sex determinants of hyper-responsiveness to SU5416 alone producing severe pulmonary arterial hypertension in a sub-strain of Sprague Dawley rats
• Presented at 2016 PVRI World Congress; 2015 American Heart Association Scientific Sessions; 2015 American Thoracic Society meeting; and 2015 Canadian Cardiovascular Congress
• Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2015; Canadian Journal of Cardiology, 2015; and Circulation, 2015

Marshaleen Henriques-Forsythe, MD
Morehouse School of Medicine
Atlanta, GA
Mentors: Vincent Bond, PhD and Harrison Farber, MD
Project Title: The prevalence and pathogenesis of HIV-associated pulmonary arterial hypertension among underserved urban populations
• Presented at 2016 American Thoracic Society Conference

Alan R. Morrison, MD, PhD
Yale University
New Haven, CT
Mentor: Hyung J. Chun, MD
Project Title: Development of microRNA-based therapeutic strategies for pulmonary arterial hypertension

Uyen T. Truong, MD
Children’s Hospital Colorado
Denver, CO
Co-investigator: Robin Shandas, PhD
Mentor: Dunbar Ivy, MD
Project Title: Non-invasively derived vascular and ventricular markers predict invasively derived hemodynamic data in children with pulmonary hypertension
• Presented at 2016 American Thoracic Society Conference; 2016 UCSF Pulmonary Hypertension Conference; and 2015 American Heart Association meeting
Overview of ENTELLIGENCE Awards

2015 Award Winners

From left: Ketul R. Chaudhary, PhD, Marshaleen Henriques-Forsythe, MD, Uyen T. Truong, MD, and Alan R. Morrison, MD, PhD
Overview of ENTELLIGENCE Awards

Awarded 2014

Evan L. Brittain, MD, MSCI
Vanderbilt University Medical Center
Co-Investigator: Thomas J. Wang, MD
Mentor: Anna R. Hemnes, MD
Project Title: Dysregulation of lipid metabolism and right ventricular function in pulmonary arterial hypertension
• Presented at 2016 American Society of Clinical Investigation annual meeting and 2015 American Heart Association Scientific Sessions
• Published in Circulation, 2016 and American Journal of Respiratory and Critical Care Medicine, 2016; Accepted for publication in Pulmonary Circulation, 2016

Joshua M. Diamond, MD
University of Pennsylvania
Co-Investigator: Harold I. Palevsky, MD
Mentor: Steven M. Kawut, MD, MS
Project Title: Clinical and biomarker risk evaluation of pulmonary hypertension in lung transplantation

R. Blair Dodson, PhD
University of Colorado Denver Anschutz Medical Center
Mentor: Steven H. Abman, MD
Project Title: Intrauterine hemodynamic stress mechanisms of fetal pulmonary vascular injury
• Presented at 2014 and 2015 Pediatric Academic Societies meetings
• Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2014

Clyde J. Wright, MD
University of Colorado School of Medicine and Children’s Hospital Colorado
Mentor: Kurt R. Stenmark, MD
Project Title: Role of macrophage ET1 expression in the pathogenesis of persistent pulmonary hypertension of the newborn
• Presented at 2015 Society for Pediatric Research Annual Meeting; 2015 Western Society for Pediatric Research Annual Meeting; and 2014 Neonatal Cardiopulmonary Biology Young Investigators Forum
• Published in Journal of Immunology, 2015
Overview of ENTELLIGENCE Awards

Awarded 2013

**Harry Karmouty-Quintana, PhD**  
The University of Texas Health Science Center at Houston  
*Mentor:* Michael R. Blackburn, PhD  
**Project Title:** The role of hyaluronan in pulmonary hypertension associated with idiopathic pulmonary fibrosis (IPF)  
- Presented at 2015 and 2016 American Thoracic Society Conferences; 2015 European Respiratory Conference; and 2014 Keystone Symposium on Molecular and Cellular Biology  
- Published in American Journal of Respiratory Cell and Molecular Biology, 2016 and FASEB Journal, 2015

**Michael L. O’Byrne, MD**  
Children’s National Heart Institute  
**Co-Investigators:** Brian D. Hanna, MD, PhD; Steven M. Kawut, MD, MS; and Russell T. Shinhara, PhD  
*Mentor:* Jonathan J. Rome, MD  
**Project Title:** Adverse outcomes associated with cardiac catheterization in children with pulmonary arterial hypertension  
- Published in American Heart Journal, 2015; American Journal of Cardiology, 2015; Catheterization Cardiovascular Intervention, 2015; Journal of the American College of Cardiology, 2015; Congenital Heart Disease, 2014; Journal of Thoracic and Cardiovascular Surgery, 2014; and Pediatric Cardiology, 2014

**Tien Peng, MD**  
UCSF School of Medicine  
*Mentor:* Edward Morrisey, PhD  
**Project Title:** The role of sonic hedgehog (Shh) signaling in pulmonary arterial hypertension  
- Published in Nature, 2015

**Keivan Zandinejad, MD**  
Case Western Reserve University School of Medicine  
*Mentor:* Jonathan S. Stamler, MD  
**Project Title:** S-Nitrosylation therapy to treat hypoxia-induced pulmonary arterial hypertension
Overview of ENTELLIGENCE Awards

Awarded 2012

Eileen Bauer, PhD
University of Pittsburgh School of Medicine
Co-Investigator: Stephen Tomlinson, PhD
Mentors: Philip M. Bauer, PhD and Timothy R. Billiar, MD
Project Title: Complement activation as a novel mechanism of endothelial activation in PH

Joshua P. Fessel, MD, PhD
Vanderbilt University Medical Center
Mentor: James D. West, PhD
Project Title: The role of sirtuins and lysine acetylation in pulmonary arterial hypertension
  • Presented at 2013 American Thoracic Society Conference
  • Published in American Journal of Respiratory and Critical Care Medicine, 2013; American Journal of Respiratory Cell and Molecular Biology, 2013; and Pulmonary Circulation, 2013
  • Book chapter published in Pulmonary Hypertension, Basic Science to Clinical Medicine, 2016

Kenny Schlosser, PhD
Ottawa Hospital Research Institute
Mentor: Duncan J. Stewart, MD
Project Title: Role of extracellular circulating microRNAs in idiopathic pulmonary arterial hypertension
  • Presented at 2013 and 2014 American Thoracic Society Conferences and 2012 American Heart Association meeting
  • Published in Chest, 2015 and American Journal of Respiratory and Critical Care Medicine, 2013

Kelly J. Shields, PhD
Allegheny Health Network
Co-Investigator: Joseph M. Ahearn, MD
Mentor: Raymond L. Benza, MD
Project Title: The role of perivascular adipose tissue in pulmonary arterial hypertension
  • Presented at 2014 American Thoracic Society Conference
Overview of ENTELLIGENCE Awards

Awarded 2011

**Jana Bagarova, PhD**
Massachusetts General Hospital and Harvard Medical School  
**Mentor:** Paul Yu, MD, PhD  
**Project Title:** BMP9-mediated regulation of endothelin-1 expression in vascular endothelial cells  
• Presented at 2011 American Heart Association meeting

**Marco Mura, MD, PhD**
Western University of Canada  
**Co-Investigator:** Marc de Perrot, MD, MSc  
**Mentor:** John Granton, MD  
**Project Title:** Osteopontin in idiopathic pulmonary arterial hypertension, a biomarker and therapeutic target  
• Presented at 2013 International Society for Heart & Lung Transplantation Annual Meeting and 2013 Canadian Respiratory Conference  
• Published in PLoS One, 2014 and Chest, 2012

**Salah Najm, MD**
University Hospitals, Case Medical Center  
**Mentor:** Kingman Strohl, MD  
**Project Title:** Vascular reactivity in response to acute hypoxia: defining features and mechanisms  
• Presented at 2012 American Thoracic Society Conference

**Yon K. Sung, MD**
Stanford University School of Medicine  
**Mentor:** Mark Nicolls, MD  
**Project Title:** The role of antibodies in the pathogenesis of pulmonary arterial hypertension
Overview of ENTELLIGENCE Awards

Awarded 2010

Eric D. Austin, MD, MSCI  
Vanderbilt University School of Medicine  
Mentor: James E. Loyd, MD  
**Project Title: Sex hormone abnormalities in pulmonary arterial hypertension**  
- Published in Circulation, 2016; Pulmonary Circulation, 2011 and 2013; and Biology of Sex Differences, 2012  

Angela V. Ghatnekar, PhD  
Medical University of South Carolina  
Mentor: Richard M. Silver, MD  
**Project Title: The role of GATA-6 in pulmonary arterial hypertension**  
- Published in Circulation, 2016; Pulmonary Circulation, 2011 and 2013; and Biology of Sex Differences, 2012  

Jason Gien, MD  
University of Colorado School of Medicine  
Mentor: Steven H. Abman, MD  
**Project Title: ET-1-Rho-kinase interactions in the pathogenesis of neonatal pulmonary hypertension**  
- Presented at 2010, 2011, and 2013 Pediatric Academic Societies meetings  

Michael J. Passineau, PhD  
Drexel University College of Medicine  
Mentor: Raymond L. Benza, MD  
**Project Title: Gene therapy to drive endogenous biosynthesis of prostacyclin**  
- Presented at 2012 American Society of Gene and Cell Therapy Annual Meeting  
- Published abstract: Molecular Therapy Supplement, 2012

Michael York, MD  
Boston University Medical Campus  
Mentor: Harrison Farber, MD  
**Project Title: dsRNA stimulates toll-like receptor-3 and increases endothelin-1 production by pulmonary artery endothelial cells**  
- Published in Current Opinion in Rheumatology, 2011; Expert Review in Clinical Immunology, 2011; Annals of the Rheumatic Diseases, 2010 and 2011; and Journal of Investigative Dermatology, 2010
Overview of ENTELLIGENCE Awards

Awarded 2009

**Daniel J. Kass, MD**
University of Pittsburgh
Co-Investigator: Hunter C. Champion, MD, PhD
Mentor: Mark Gladwin, MD
Project Title: Targeting the MetAP2 pathway in pulmonary arterial hypertension
- Presented at 2010 and 2011 American Thoracic Society Conferences
- Published in PLoS One, 2012

**Sean E. McLean, MD**
University of North Carolina at Chapel Hill School of Medicine
Mentor: Cam Patterson, MD, MBA
Project Title: Smooth muscle cell related vascular remodeling in pulmonary hypertension in congenital diaphragmatic hernia

**Alexander R. Opotowsky, MD, MPH**
Boston Children’s Hospital
Mentor: Michael J. Landzberg, MD
Project Title: The epidemiology and determinants of hospitalization for pulmonary hypertension in the United States
- Presented at 2013 American College of Cardiology meeting

**Michael E. Yeager, PhD**
University of Colorado School of Medicine
Mentor: Dunbar Ivy, MD
Project Title: Circulating mesenchymal precursors in severe PAH and the role of endothelin-1 in their recruitment and differentiation into fibrocytes
- Published in Chest, 2012 and European Respiratory Journal, 2012
Overview of ENTELLIGENCE Awards

Awarded 2008

Gaurav Choudhary, MD
Alpert Medical School at Brown University
Mentor: James Klinger, MD
Project Title: Role of endothelin-induced PKC delta activation in right ventricular hypertrophy

Hyung J. Chun, MD
Yale University School of Medicine
Mentor: Thomas Quertemous, MD
Project Title: Role of the apelin-APJ pathway in endothelin-1 signaling and pulmonary arterial hypertension
• Presented at 2009 American Heart Association meeting and 2009 American Thoracic Society Conference
• Published in Circulation, 2015; Circulation Research, 2013; Nature Medicine, 2013; and Arteriosclerosis, Thrombosis, and Vascular Biology, 2011

Scott D. Halpern, MD, PhD
Perelman School of Medicine at the University of Pennsylvania
Mentor: Brian Strom, MD
Project Title: Racial differences in responsiveness to endothelin receptor antagonists in pulmonary arterial hypertension

Sayyed A. Hamidi, MD
State University of New York, Stony Brook
Mentor: Sami I. Said, MD
Project Title: A new combination therapy for pulmonary arterial hypertension: bosentan and VIP
• Presented at 2010 American Thoracic Society Conference; 2010 Aspen Lung Conference; and 2010 Annual Congress of European Respiratory Society
• Published in Respiratory Research, 2011
• Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2010 and European Respiratory Journal Supplement, 2010

Sanjiv Shah, MD
Northwestern University Medical Center
Mentor: John Varga, MD
Project Title: Genetic risk factors for connective tissue disease (CTD)-associated pulmonary arterial hypertension (PAH)
• Published in Arthritis Research & Therapy, 2015; Journal of Investigative Dermatology, 2013; Clinical and Experimental Rheumatology, 2012; and Current Rheumatology Reports, 2009

Venkataramana Sidhaye, MD
Johns Hopkins University
Mentor: Larissa Shimoda, PhD
Project Title: Endothelin-1 mediated pulmonary smooth muscle migration is mediated by AQP1
• Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2012

Ari L. Zaiman, MD, PhD
Johns Hopkins University
Mentor: Hal Dietz, MD
Project Title: Role of endothelin abrogation of TGF signaling in the vascular endothelium attenuates hypoxia induced pulmonary hypertension
• Presented at 2010 American Thoracic Society Conference
Overview of ENTELLIGENCE Awards

Awarded 2007

Yabing Chen, PhD
UAB School of Medicine
Mentor: Raymond Benza, MD
Project Title: PAI-1 regulates vascular remodeling in hypoxia-induced pulmonary hypertension

Christopher Fiack, MD
The Queens Medical Center
Mentor: Harrison Farber, MD
Project Title: Pulmonary hypertension due to left ventricular dysfunction

Anna R. Hemnes, MD
Vanderbilt University School of Medicine
Mentor: John Newman, MD
Project Title: The role of endothelin-1 in right ventricular response to pressure overload
  • Presented at 2008 American Thoracic Society Conference

Jeffrey C. Horowitz, MD
University of Michigan Health System
Mentor: Victor J. Thannickal, MD
Project Title: Regulation of myofibroblast resistance to apoptosis by endothelin-1
  • Published in American Journal of Respiratory Cell and Molecular Biology, 2009

Meredith A. Preuss, PhD
University of Alabama at Birmingham
Mentor: David Curiel, MD
Project Title: Downstream redox regulation of endothelin B receptor in the pulmonary endothelium
  • Published in Virology, 2013 and The Open Gene Therapy Journal, 2008

Olga Rafikova, MD, PhD
Georgia Health Sciences University
Mentor: Steven P. Tofovic, MD, PhD
Project Title: Protein nitration and anti-remodeling effects of endothelin receptor antagonists in pulmonary hypertension
  • Presented at 2010 European Respiratory Society Congress; 2009 American Thoracic Society Conference; and 2009 Pittsburgh International Lung Conference
  • Published in Free Radical Biology and Medicine, 2013
  • Published abstracts: European Respiratory Society Congress, 2010; American Thoracic Society Conference, 2009; and Pittsburgh International Lung Conference, 2009

Megha H. Talati, PhD
Vanderbilt University
Mentor: Barbara Meyrick, PhD
Project Title: Effect of BMPR2 mutation in FPAH on ET-1 and ET-1 receptors and Smad/MAPK activation by ET-1 receptors in lung ECs and PASMCs in the mouse model of PAH
  • Presented at 2009 American Thoracic Society meeting
  • Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2010

Yerem Yeghiazarians, MD
UCSF School of Medicine
Mentor: Teresa DeMarco, MD
Project Title: Effect of endothelin receptor blockade on circulating endothelial microparticle levels in patients with pulmonary hypertension
Overview of ENTELLIGENCE Awards

Awarded 2006

Joel Glasgow, PhD
UAB School of Medicine
Mentor: David Curiel, MD
Project Title: Gene delivery for pulmonary hypertension

Zhigang Hong, MD, PhD
University of Chicago
Mentor: Kenneth Weir, MD
Project Title: Endothelin-induced increase in pulmonary vascular smooth muscle calcium; the role of calcium channels
  • Published in European Respiratory Journal, 2008
  • Published abstracts: Circulation, 2008 and 2010; American Thoracic Society (ATS) meeting, 2007; and ATS International Conference, 2007

Peter Oishi, MD
UCSF School of Medicine
Mentor: Jeffrey Fineman, MD
Project Title: Endothelin-1 reactive oxygen species interactions in pulmonary hypertension
  • Presented at 2007 Pediatric Academic Societies meeting
  • Published in American Journal of Physiology - Heart and Circulatory Physiology, 2008
  • Book chapter published in Congenital Diseases in the Right Heart, 2009

Rajni Rao, MD
UCSF School of Medicine
Mentor: Yerem Yeghiazarians, MD
Project Title: Quantitative and qualitative properties of endothelial progenitor cells in patients with pulmonary hypertension
  • Presented at 2007 International Society of Heart and Lung Transplantation meeting

Giuseppe Valacchi, PhD
University of Ferrara
Mentors: Carol Cross, MD and Gian Paolo Pessina, Professor
Project Title: Does tocopherol homeostasis play a role in endothelin mediated endothelial dysfunction?

Roham Zamanian, MD
Stanford University Medical Center
Mentor: Ramona Doyle, MD
Project Title: The effect of endothelin A and B antagonism on insulin resistance and outcomes in patients with pulmonary arterial hypertension
Impact of mitochondrial heat shock protein 90 inhibition in pulmonary arterial hypertension

Introduction:
Pulmonary arterial hypertension (PAH) is a fatal disease characterized by a cancer-like proliferative, apoptosis-resistant phenotype of pulmonary arterial smooth muscle cells (PASMCs). There is currently no effective treatment. A better understanding of the pathogenesis of PAH along with the identification of targets suppressing abnormal growth of PASMCs are needed.

Background:
Pulmonary arterial hypertension (PAH) is defined as an increase in pulmonary artery pressure ≥25mmHg at rest. Several groups have demonstrated that PAH is a disease of excess proliferation and impaired apoptosis of pulmonary arterial smooth muscle cells (PASMCs) similar to neoplasia. Although the fundamental cause remains elusive, many cancer predisposing abnormalities occur, including endothelial injury/dysfunction, decreased expression of the K+ channel (Kv1.5), metabolic shift towards a glycolytic metabolism (Warburg effect), transcription factor activation (HIF-1α, STAT3, NFAT), and overexpression of proto-oncogenes (Survivin, Pim-1). Together, these abnormalities create a cancer-like, proliferative, apoptosis-resistant phenotype. These results bring an emerging paradigm in PAH pathology and could give the possibility to combine the therapeutic strategies used in cancer to treat PAH. Hsp90 is a key member of the quality control machinery present in the cell, implicated in the folding and maintenance of newly translated proteins or to the degradation of misfolded and destabilized proteins. In tumor cells, up-regulation of Hsp90 orchestrates a broad cell-survival program. Because of its multiple roles as a cancer gene, and the potential “drugability” of its ATPase pocket, Hsp90 has been pursued for novel cancer therapeutics, and a small molecule Hsp90 antagonist has entered clinical testing in cancer patients. In direct connection with PAH, Hsp90 stabilizes Survivin, PIM-1, and HIF-1α, which play deleterious roles in vascular remodeling. Interestingly, mitochondrial Hsp90 controls central metabolic networks in tumor cells. The ability of HSP90 inhibitor to affect multiple oncogenic pathways simultaneously is a unique and therapeutically attractive feature of this compound in PAH.

Hypothesis and Objectives:
Based on preliminary results demonstrating that Hsp90 is up-regulated in PAH, we hypothesize that increased Hsp90 expression is implicated in PAH. Using a translational and multidisciplinary approach, our overall objective is to mechanistically investigate the role of Hsp90 in PAH pathogenesis and uncover a promising new therapeutic target.

Specific Aim 1:
In AIM1, we will test the hypothesis that Hsp90 contributes to PAH-PASMC proliferation, apoptosis resistance, and migration.

Specific Aim 2:
In AIM2, we will identify the mechanisms whereby Hsp90 mediates PASMC proliferation and cell survival.

Specific Aim 3:
In AIM3, we will investigate the therapeutic potential of Hsp90 inhibition in vivo.
2016 ABSTRACTS

Vitaly O. Kheyfets, PhD
University of Colorado Denver
Denver, CO

Inter-ventricular decoupling is an overlooked contributor to right ventricular myocardial stress and dysfunction in pediatric pulmonary hypertension

Introduction:
Pulmonary hypertension (PH) in children is a complex disease with multiple underlying etiologies, characterized by progressive pulmonary arterial (PA) and right ventricular (RV) dysfunction. Ongoing work has been successful in elucidating key events in the RVPA axis, but more is needed to investigate the role of the left ventricle (LV).

Background:
Previous studies have shown that LV contraction can generate almost 70% of the systolic pressure and 80% of the pulmonary flow in the passive RV of dogs, while the role of the RV is negligible for generating LV pressure. Therefore, the RV is relying in the contractile energy transfer from the LV to aid in perfusing the lungs, which is concerning given that echocardiographic evaluation of the LV has demonstrated reduced LV strain and strain-rate in pediatric PH patients. Thus, it is likely that PH patients could benefit both from therapies and diagnostic markers that target the RV-LV axis. Yet systematic studies to evaluate the underlying mechanical coupling between these two pumps in the face of hydraulic loading of one pump in the pediatric pulmonary hypertension population have not been performed. Further, connecting such mechanistic information to functional and biological markers that may provide early indicators of abnormal RV-LV coupling has likewise not been undertaken.

Hypothesis and Objectives:
The overall hypothesis of this study is that decreased LV torsion-rate directly compromises contractile function in the overloaded RV, which is accompanied by abnormal myocardial wall stress and biomarker expression.

Specific Aim 1:
Identify the role of LV torsion-rate in PAH using tagged MRI imaging and hemodynamics: 1.1. Using tagged MRI, compare maximum LV torsion-rate between normotensive children (n = 15, recruited) and children with PAH (n = 10 expected recruitment). 1.2. Associate LV torsion-rate with RV contractility and function in PAH.

Specific Aim 2:
Establish cause-and-effect between LV torsion-rate and: RV contractility and myocardial stress: 2.1. Develop a patient-specific mathematical model of the cardiopulmonary complex to compute myocardial wall stress for each patient. 2.2. Repeat Aim 2.1 for each PAH subject under normal LV torsion-rate, and compute change in RV contractility and wall stress.

Specific Aim 3:
Measure serum NT-proBNP and identify circulating miRNAs that are associated with abnormal LV torsion-rate and myocardial wall stress (n = 10 patients; NT-proBNP and 765 miRNAs per patient will be measured).
PDE9A in right ventricular and pulmonary vascular remodeling

Introduction:
Augmentation of cyclic guanosine monophosphate (cGMP) signaling via phosphodiesterase (PDE) inhibition is a cornerstone of therapy in PAH. Available PDE inhibitors require endogenous nitric oxide (NO), which has limited bioavailability in PAH. This application aims to elucidate the role of an NO-independent PDE (PDE9A) in a murine PH model.

Background:
The intracellular second messenger cGMP is critical for endothelial, vascular smooth muscle, and cardiac myocyte function. In the RV and pulmonary circulation, NO and natriuretic peptides are the two primary endogenous activators of cGMP. PDE activity promotes cGMP catabolism, and PH promotes PDE5 expression (and cGMP inhibition) in the RV and pulmonary vasculature. PDE5 inhibitors (sildenafil, tadalafil) have vasodilatory and anti-proliferative effects in the lung and inotropic effects in the RV. However, reduced NO bioavailability may limit efficacy in some patients. We have intriguing new preliminary data that an alternative, NO-independent PDE (PDE9A) is up-regulated in the RV during myocardial remodeling in a murine PH model (chronic hypoxic PH; CH-PH). Our coinvestigator, Dr. David Kass, recently demonstrated that PDE9A is up-regulated in the left ventricle during hypertrophy and failure. Furthermore, PDE9A specifically inhibited natriuretic peptide-cGMP signaling (not NO-cGMP) in cardiac myocytes, and PDE9A inhibition prevented pressure-overload induced left ventricular remodeling independent of NO. The role of PDE9A in RV and pulmonary vascular remodeling has not been explored. Given the critical role of cGMP-dependent signaling in myocyte and pulmonary vascular homeostasis, we believe the elucidation of an NO-independent regulatory pathway may yield significant therapeutic promise.

Hypothesis and Objectives:
We hypothesize that PDE9A regulates RV and pulmonary vascular remodeling via cGMP signaling in the CH-PH model. This proposal aims to use advanced molecular, morphological, and physiological methods to elucidate the role of PDE9A activity on CH-PH induced cGMP signaling, RV and pulmonary vascular remodeling.

Specific Aim 1:
To determine whether PDE9A regulates RV and lung cGMP signaling during CH-PH. We will expose PDE9A-/- mice and wild-type littermates to CH-PH for 1 and 3 weeks. We will measure and compare PDE9A expression and activity, cGMP, and protein kinase G activity (PKG-1; downstream effector kinase) in RV and lung samples.

Specific Aim 2:
To measure the effects of PDE9A deficiency on CH-PH induced RV and pulmonary vascular remodeling. Through a combination of stereological morphometry and RV pressure-volume loops, we will compare pulmonary vascular and RV remodeling between PDE9A-/- mice and wild-type littermates after 1 and 3 weeks of CH-PH.
Iron deficiency and hypoxic signaling in pulmonary hypertension

Introduction:
There is increasing evidence of importance of iron in pulmonary arterial hypertension (PAH); Iron deficiency is associated with worse outcomes, and current trials are addressing the benefit of iron repletion (clinical trials.gov #NCT01447628). However, how iron affects the pulmonary circulation remains unclear.

Background:
Iron deficiency is present in up to 63% of patients with idiopathic PAH, and correlates with worsened functional class and mortality independent of anemia – though a causative relation has not been demonstrated. Small clinical trials of iron supplementation in PAH have demonstrated both a small increase and no change in functional capacity. Further, published studies in animal models of pulmonary hypertension (PH) have shown conflicting improvement or worsening of pulmonary artery pressures and vascular remodeling with induction of iron deficiency. The discordant findings highlight the complexity of the role of iron in the pathobiology of PAH. Iron is a cofactor for over 1000 enzymes, a source of oxidative stress, and essential for cellular proliferation. Further, iron has the ability to drive and inhibit hypoxia inducible factor (HIF) signaling – strongly implicated in the pathobiology of PH – through iron regulatory protein and prolyl hydroxylase (PHD)-mediated mechanisms. Utilizing the hypoxic mouse model of PH, I have preliminarily identified a significant protective effect of iron deficiency on the development of PH. Further, I have found that pulmonary HIF transcriptional activity is not altered by iron depletion. Rather, iron depletion leads to a robust increase in renal HIF activity with an increase in serum erythropoietin (EPO) levels – a canonical HIF target.

Hypothesis and Objectives:
I hypothesize that in murine chronic hypoxic pulmonary hypertension, iron depletion leads to increased renal HIF availability via decreased PHD activity, resulting in increased production of EPO. This EPO then attenuates pulmonary hypertension through increased eNOS signaling in the pulmonary vasculature.

Specific Aim 1:
To determine that low iron availability in chronic hypoxic PH increases renal HIF2 stability via decreased PHD activity, resulting in increased serum EPO levels.

Specific Aim 2:
To determine that blocking circulating EPO, by administering exogenous soluble EPO receptor, results in loss of protective effect of iron deficiency in PH.

Specific Aim 3:
To determine that EPO signaling in pulmonary vascular endothelial cells results in activation of eNOS signaling.
Introduction:
The project for which we request funding will focus on the implication of PI3K/akt/mTOR signaling pathway in the development of pulmonary hypertension (PH). We expect completion of the proposed studies will provide novel insights into the understanding of pathogenesis of PH, which may lead to novel therapeutic targets for PH.

Background:
Pulmonary arterial hypertension (PAH) is a rare but progressive and deadly disease caused by functional and structural changes in the pulmonary vasculature, which lead to an increase in pulmonary vascular resistance (PVR). Pulmonary vascular remodeling or concentric vascular wall thickening of small pulmonary arteries and arterioles, due partially to enhanced proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs), is a major cause for elevated PVR in patients with PAH and chronic hypoxia-induced pulmonary hypertension (HPH). Multiple cellular and molecular mechanisms have been demonstrated to contribute to the development and progression of pulmonary vascular remodeling; however, the specific sequence of events involved in the enhanced PASMCs proliferation in pulmonary hypertension remains unclear. The PI3K-Akt-mTOR pathway is one of the fundamental intracellular signaling cascades that communicate extracellular mitogenic signals to the nuclear transcriptional machinery that induces cell proliferation and protein synthesis. Our previous studies demonstrate that unique Akt isoforms have distinctive effects in the development of HPH and that Akt1 but not Akt2 is essential for pulmonary vascular remodeling. We have also shown the importance of the Akt1/mTOR pathway in which increased levels of PTEN, an upstream repressor, or specific KO of downstream mTOR in PASMC, can significantly attenuate the development and progression of HPH. The Akt/mTOR pathway will continue to be a focus of research aimed at determining factors that affect pulmonary vascular remodeling, and focusing on specific isoforms or targets of this pathway may lead to novel therapeutic targets for pulmonary hypertension.

Hypothesis and Objectives:
Since mTOR associates with different proteins (Raptor or Rictor) to form two functional macromolecular complexes (mTORC1 and mTORC2), we propose that mTORC1 and mTORC2 play differential roles in the development of hypoxia-induced pulmonary hypertension and dual inhibition of mTOR1/2 complexes attenuates hypoxic vasoconstriction and the development of pulmonary hypertension.

Specific Aim 1:
To determine the roles of mTORC1 and mTORC2 in the cell proliferation, migration, smooth muscle cells phenotypic switching under hypoxia conditions, and PAECs endothelial-mesenchymal transition using pharmacologic and genetic approaches.

Specific Aim 2:
To investigate whether mTORC1/2 complexes regulate hypoxic pulmonary vasoconstriction (HPV) and the development of vascular remodeling in HPH models using PASMC or PAEC conditional KO mice.

Specific Aim 3:
To determine whether dual inhibition of mTORC1/2 complexes prevent and reverse the development of HPH, using inhibitors targeting the Akt/mTORC1/2 signaling pathway.
Introduction:
Pulmonary arterial hypertension (PAH) is a progressive disease with unclear etiology characterized by increases in mean pulmonary arterial pressure (>25 mmHg) leading to right ventricular hypertrophy and heart failure, and ultimately death. While pharmacotherapy can slow the progression of the disease, there is no cure.

Background:
PAH is a multifactorial disease with a strong genetic component. Mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene account for a substantial proportion of hereditary and sporadic disease, but the penetrance is low (~20%). A multitude of environmental factors have also been implicated in PAH, including exposure to toxins, anorexigens, high shear stress and viral infections; however, the exact pathobiology still remains unclear. Recently, a new animal model has been introduced that better reproduces the salient pathological features of human PAH, involving the injection of a single dose of the VEGFR2 antagonist, SU5416 (SU), followed by a 3-week exposure to chronic hypoxia (CH). SU is believed to cause lung endothelial cell apoptosis that, together with CH as a “second hit,” results in the emergence of growth dysregulated, quasi-neoplastic vascular cells that form characteristic plexiform-like arterial lesions. Our lab has studied strain differences in the SU/CH model of PAH. Interestingly, we observed that a specific sub-strain of the Sprague Dawley rats obtained from a Canadian supplier was hyper-responsive to SU and developed a progressive severe PAH phenotype in response to a single SU injection, even in the absence of CH. The hyper-responsive phenotype was seen in 70-75% of the male rats; whereas only 25-30% of the female rats were responsive to SU alone. Furthermore, crossing non-responsive male and female animals markedly decreased the proportion of hyper-responsiveness in the F1-generation (male: 15%; female: 0%), highly suggestive of a genetic basis for the hyper-responsive phenotype.

Hypothesis and Objectives:
We hypothesize that hyper-responsiveness to SU alone is conferred by as yet unknown genetic determinant(s). Moreover, the influence of this genetic determinant(s) is importantly modified in a sex dependent manner, likely by the action of female sex hormones.

Specific Aim 1:
To identify genetic determinant(s) in the SU hyper-responsive Sprague Dawley rats using the exome sequencing or the whole genome sequencing in collaboration with the STAR rat genome consortium. This work could uncover novel genetic factors associated with PAH.

Specific Aim 2:
To explore the role of sex hormones in modifying the SU hyper-responsive phenotype using surgical (e.g. oophorectomy/castration) or pharmacological (e.g. hormone replacement or inhibition) manipulations.

Specific Aim 3:
To explore the mechanistic relevance of “modifier” genes identified in Aim 1 in the pathogenesis and treatment of human PAH using human material (DNA, lung tissue, vascular cells and blood) available from patients with idiopathic and hereditary PAH.
The prevalence and pathogenesis of HIV-associated pulmonary arterial hypertension among underserved urban populations

Introduction:
Pulmonary arterial hypertension (PAH) is a non-infectious complication of HIV which has become increasingly important as HIV survival has increased. Although HIV is an independent risk factor for PAH, the pathogenesis of HIV-associated PAH (HIV-PAH) is largely unknown and specific prevalence data for minority populations have not been investigated.

Background:
The three-year survival for HIV-PAH is significantly higher for persons diagnosed as New York Heart Association (NYHA) functional classes I & II (~85%) compared to patients diagnosed as NYHA functional classes III & IV (~30%). Unfortunately, HIV-PAH is associated with non-specific symptoms; thus, it is often diagnosed late, leading to the need for more expensive therapeutic options and a greater health burden. African-Americans may have an escalating risk for developing HIV-PAH given the rapid increase in HIV infection among African-American women. In addition, studies suggest that the prevalence of preclinical HIV-PAH may be higher than that of clinically diagnosed HIV-PAH. If this is true, routine PAH screening should be considered for persons with HIV, given the poor prognosis of HIV-PAH when diagnosed late or left untreated. This study will use targeted screening to determine the prevalence of HIV-PAH among African-Americans in Atlanta with a view to developing screening guidelines for this high-risk population.

The HIV protein Nef has been implicated in HIV-PAH pathogenesis, but the exact mechanism is unknown. Additional studies are needed to explore the role of Nef in HIV-PAH pathogenesis among humans. This study will 1) investigate the relationship between HIV-PAH and exosomal Nef and 2) determine if exosomal Nef has predictive or prognostic value with regards to the development and progression of HIV-PAH, respectively. Knowledge gained regarding exosomal Nef’s role in HIV-PAH pathogenesis may prove useful in developing screening guidelines and may identify pathways that could be targeted to intervene in HIV-PAH pathogenesis.

Hypothesis and Objectives:
We hypothesize that: 1) targeted screening for HIV-PAH among an urban underserved community will improve the early detection of PAH; and 2) there is a relationship between HIV-PAH and the levels of exosomal Nef.

Specific Aim 1:
To determine whether targeted screening for HIV-PAH among an urban underserved community improves early detection of PAH.

Specific Aim 2:
To determine whether there is a correlation between PAH and HIV Nef-driven exosome-linked factors.
Introduction:
Pulmonary arterial hypertension is a disease characterized by the vascular remodeling of the pulmonary arterioles, leading to increased pulmonary vascular resistance and right ventricular failure. A number of microRNAs have emerged as promising therapeutic targets, but significant hurdles remain before they can be pursued in clinical studies.

Background:
Pulmonary arterial hypertension (PAH) is a disease of exceedingly high mortality with limited therapeutic modalities. Work from the Chun (mentor) laboratory and others have identified key microRNAs involved in the disease pathogenesis, including miR-424, miR-503, miR-204, miR-130, miR-301, miR-21, miR-145, and miR-124. Although these studies have all identified novel pathways in experimental models with strong translational applicability to human disease, a number of limitations prevent advancement of a microRNA based therapeutic strategy in PAH. First, there is currently no standardized delivery strategy for the microRNA mimics or antagonists to the pulmonary vascular cells. Second, although each of these studies has demonstrated varying degrees of therapeutic efficacy for the specific microRNAs of interest, a direct comparison of efficacy using a standardized delivery technique and disease model has never been investigated. The current proposal will identify an effective delivery strategy for oligonucleotide based therapy to the pulmonary vasculature. It will then utilize this strategy to compare the efficacy of targeting candidate microRNAs known to be involved in the pathogenesis of PAH, to identify the best therapeutic targets for development of early clinical trials. We will utilize novel, vascular targeting nanoparticle based delivery strategies, in conjunction with robust experimental rodent models of pulmonary hypertension, to pursue these studies.

Hypothesis and Objectives:
We hypothesize that a nanoparticle based approach for delivery of microRNA mimics or antagonists is an effective strategy for treatment of pulmonary arterial hypertension. We will determine the most effective delivery strategy and identify the strongest microRNA candidate(s) with the goal of developing early clinical studies.

Specific Aim 1:
Develop and identify the optimal nanoparticle composition and delivery strategy for targeting oligonucleotides to the pulmonary vasculature. We will test the efficacy of modified nanoparticle compositions to achieve selective delivery of microRNA mimics or antagonists in vitro to primary cells of the pulmonary vasculature.

Specific Aim 2:
Identify the microRNA targets with the most effective therapeutic efficacy in rescuing preclinical models of pulmonary hypertension. We will utilize the optimal nanoparticle composition identified in Specific Aim 1 to test the efficacy of targeting multiple microRNA candidates in vivo, using the monocrotaline and the SU-5416/hypoxia models of pulmonary hypertension.

Specific Aim 3:
Evaluate the feasibility of combining microRNA targets to augment therapeutic efficacy. We will determine if a combination of the best microRNA mimics/antagonists (identified in Aim 2) can achieve an additive or synergistic effect to augment therapeutic efficacy.
Non-invasively derived vascular and ventricular markers predict invasively derived hemodynamic data in children with pulmonary hypertension

Introduction:
Prognosis in childhood pulmonary hypertension (PH) remains poor. Research efforts in pediatrics are complicated by the disease's multifactorial nature and potential risks posed on subjects. The single, standard diagnostic measure is pulmonary vascular resistance. This is only one of many factors predictive of mortality and does not reflect ventricular dysfunction.

Background:
Ventricular-vascular coupling (VVC) is critical in understanding the interaction between the pulmonary vasculature and the myocardial function in pulmonary hypertension (PH). The gold standard for determining VVC is cardiac catheterization. The invasive nature of this procedure has led clinicians to look towards non-invasive imaging modalities. Recently, studies have demonstrated that VVC can be estimated with CMR in PH. This can reduce radiation and anesthesia exposure to PH children requiring longitudinal monitoring. We have previously shown that VVC by CMR has high predictability for vascular acute reactivity in pediatric PH. In a more recent study, we prospectively recruited 46 pediatric PH and 26 normotensive controls to evaluate VVC by CMR. Bivariable analysis indicated significant correlation between VVC and current WHO score (p=0.02), WHO score progression over the past year (p<0.01), and the current use of intravenous/subcutaneous therapy (the most intensive forms of PH therapy) (p<0.01). The subject group in this recent study is small and is a significant limitation to the interpretation of these data. That, however, does not negate the importance of our findings and is the stepping stone to posing the question for clinicians: can serial catheterization in children with PH be avoided if non-invasive imaging can provide accurate and adequate cardiovascular data for the management of these patients? This can potentially open wide the door for facilitating increased research in a vulnerable population that is unique from the adult population in whom the majority of PH research has been done.

Hypothesis and Objectives:
Furthermore, CMR-derived ventricular-vascular coupling correlates with hemodynamic data by cardiac catheterization and 0-D computational model. VVC is predictive of future clinical deterioration, including WHO progression and escalation of medicinal needs.

Specific Aim 1:
VVC correlated with hemodynamic data. VVC has been shown in adults, excluding those with congenital heart disease, to correlate well with hemodynamic data, including pulmonary vascular resistance. Our aim is to evaluate VVC with hemodynamic data, including PVR, pulmonary vascular stiffness, transpulmonary gradient, and right ventricular end-diastolic pressure.

Specific Aim 2:
VVC by CMR in PH children has predictive value in disease progression and outcomes. We will correlate VVC to future WHO classification progression, future medicinal requirements, and progression of 6-minute walk. This will determine predictive value of VVC and its role in understanding maladaptive ventricular vascular remodeling in pediatric PH.

Specific Aim 3:
VVC by CMR correlated with a validated 0-D right ventricular-pulmonary artery computational model to assess differences in right ventricular-pulmonary response to altered hemodynamics. The 0-D model will allow the reconstruction of 2 pressure-volume loops: one at baseline and one that corresponds to a challenge condition, similar to the catheterization laboratory.
Role of macrophage ET1 expression in the pathogenesis of persistent pulmonary hypertension of the newborn

Background
Persistent pulmonary hypertension of the newborn (PPHN) affects up to 6 per 1000 live births and is the most common cause of cardiopulmonary failure in term newborns. Despite optimal medical management with inhaled nitric oxide (iNO), 40-45% of patients with PPHN die or need extracorporeal membrane oxygenation therapy. Currently, no safe and effective alternative therapies for babies with PPHN unresponsive to iNO exist. Multiple conditions manifest clinically as PPHN and it is likely that diverse molecular mechanisms underlie their pathophysiology. Chorioamnionitis and perinatal infection cause 20-30% of cases of PPHN and histologic evidence of antenatal infection is an independent risk factor for severity of disease. While it is clear that antenatal exposure to inflammatory stress is associated with PPHN, the specific cells, signaling pathways and molecular mediators responsible for this response are poorly understood. Thus, no specific therapies targeting the unique mechanisms linking perinatal inflammation and PPHN have been identified.

Whether inflammatory stress-induced endothelin-1 (ET-1) expression contributes to the pathophysiology of persistent pulmonary hypertension of the newborn remains largely unexplored. Neonates with PPHN have elevated serum ET-1 levels; however, this finding has not been separately reported in the subset of patients exposed to chorioamnionitis. The role of endothelin receptor blockers has never been tested in animal models of inflammation-associated neonatal pulmonary hypertension. These gaps in our knowledge are further reflected in recent reviews of PPHN that fail to discuss any potential mechanisms that link inflammation, ET-1 expression and the pathophysiology of PPHN. These gaps may explain why the safety and efficacy of endothelin receptor blockade has been reported in less than 30 neonatal patients with pulmonary hypertension and never specifically for patients with inflammation-associated PPHN. Demonstrating that ET-1 contributes to the pathophysiology of inflammation-associated PPHN would support using endothelin receptor blockers in this specific subset of patients.

Although the pulmonary endothelium is considered to be the primary source of ET-1, macrophages secrete ET-1 in response to a variety of pro-inflammatory stimuli. The macrophage has never been investigated as the potential source of pathologic ET-1 in patients with PPHN. As monocytic and macrophage function is developmentally regulated, investigation to determine the relationship between antenatal inflammatory stress and macrophage derived ET-1 expression necessitates the study using animal models during critical developmental windows.

Importantly, the rate-limiting step of ET-1 bioavailability is gene transcription. Therefore, transcriptional activation of ET-1 in pulmonary macrophages may represent a potential therapeutic target in cases of inflammation-associated PPHN. Importantly, the transcriptional regulation of ET-1 in response to LPS within the macrophage has not been determined. The dimeric transcription factor NFkB regulates the cellular response to inflammatory stress, and dictates the expression of many factors implicated in the pathogenesis of inflammatory lung injury. The ET-1 promoter has NFkB binding sites, and cytokine-
induced NFκB activation regulates ET-1 expression in smooth muscle and endothelial cells.\textsuperscript{35,39,45,46} Demonstrating that TLR4/NFκB signaling mediates LPS-induced ET-1 expression from macrophages in the fetal lung would identify therapeutic targets to prevent ET-1 expression.

Therefore, we hypothesized that antenatal inflammation causes PPHN in part due to NFκB regulated ET-1 expression from fetal lung macrophages (Figure 1). To test this hypothesis, I propose the following specific aims:

**Specific Aims**
1. Demonstrate that TLR4-NFκB signaling drives LPS-induced macrophage ET-1 expression.
2. Establish that antenatal inflammation induces NFκB-regulated ET-1 expression in fetal lung macrophages.
3. Test whether pharmacologic NFκB or ET receptor blockade will attenuate antenatal inflammation-induced PPHN.

**Results**
The results of the work funded by this project have recently been published in the *Journal of Immunology* (PMID 26342031). Through this project, I have established an important relationship with Dr. Stenmark. He was able to provide a letter of support for my first R01 application that was submitted in October 2015. I cannot thank the Entelligence Young Investigator Program enough for funding this work, giving me the opportunity to pursue something exciting, and help develop my research career. Of note, this published work focuses on studies performed on adult animals. Given the small amount of tissue available for isolation from the neonatal mice, we took the practical approach of starting with adult animals to ensure that we could get various assays (antibodies, qPCR primers, etc) to work. One finding led to another, and allowed us to link NFκB singaling in hepatic macrophage to increased circulating ET-1 during endotoxemia. Similar studies are now being performed in neoantal animals. Here, we will highlight what we feel are the most important findings:

1) In endotoxemic adult animals, the hepatic macrophage is the primary source of circulating ET-1 (Figure 1). Surprisingly, pulmonary ET-1 expression actually decreases during endotoxemia.

2) Increased hepatic ET-1 expression is temporally associated with NFκB activation. We showed cytosolic degradation of the NFκB inhibitory proteins IκBα and IκBβ, as well as nuclear translocation of key NFκB subunits (p50, p65 and cRel) in hepatic tissue isolated from endotoxemic mice.

3) Using both isolated primary cells (endothelial and macrophages) and cell lines (RAW 264.7 and lung microvascular endothelial cells), we showed that LPS-induced ET-1 expression occurs only in the macrophage, and that this occurs via an NFκB dependent mechanism. CHIP assay confirmed NFκB binding at the ET-1 promoter.

4) We demonstrated that LPS-induced ET-1 expression could be inhibited by selectively targeting IκBβ/NFκB signaling. This was shown using both pharmacologic (low-dose IKK inhibitors) and genetic (IκBβ overexpression) approaches (Figure 2). These findings are important as complete inhibition of NFκB signaling has been shown to have deleterious effects during septic shock.

**Conclusions**
We conclude that in the setting of endotoxemia, IκBβ/NFκB activation induces ET-1 expression in the hepatic macrophage. These findings challenge our current understanding of the mechanisms that link ET-1 and the pathogenesis of septic shock. This work has clearly defined the source and transcriptional regulation of ET-1 in response to endotoxemia and could potentially help guide therapies to improve the outcomes of patients with septic shock. By targeting specific cells and signaling pathways, clinicians may be able to target therapies to offset the detrimental effects of splanchnic, renal, and pulmonary vasoconstriction while maintaining blood pressure and improving the outcomes of patients with sepsis. Furthermore, these results provide a starting point for evaluating the role of IκBβ/NFκB induced ET-1 expression in the neonate, and how this may contribute to PPHN.
Role of macrophage ET1 expression in the pathogenesis of persistent pulmonary hypertension of the newborn (continued)

**Figure 1.** Endotoxic shock induces hepatic macrophage ET-1 expression.
(A) Serum ET-1 levels, (B) pulmonary ET-1 mRNA expression, and (C) hepatic ET-1 mRNA expression in mice exposed to LPS (50 mg/kg i.p.). Values are means ± SE (n = 6/time point). *p < 0.05 versus unexposed controls. (D and E) Representative immunofluorescence staining of (D) control and (E) LPS-exposed mouse liver. ET-1 was stained in green (a and d) and endothelial (CD31; b and c) and macrophage (F4-80; e and f) markers were stained in red. Scale bar, 20 μM. (F) Fold change in LPS-induced ET-1 mRNA expression in whole liver and isolated ihMNCs. Values are means ± SE (n = 4/time point). *p < 0.05 versus unexposed controls, †p < 0.05 versus whole liver LPS exposed. (G) Fold change in LPS-induced ET-1 expression after clodronate ablation of hepatic macrophages. Values are means ± SE (n = 6/time point). *p < 0.05 versus unexposed controls, †p < 0.05 versus LPS exposed.

**Figure 2.** The NFκB inhibitors BAY-7085 and parthenolide inhibit LPS-induced NFκB activation and the expression of downstream target genes including ET-1 in RAW 264.7 macrophages. (A) Representative Western blot showing IκBβ and IκBa in whole cell lysates from cells pretreated with BAY-7085 (5–20 mmol/l, 1 h) or parthenolide (5–20 mmol/l, 1 h) before LPS exposure (1 mg/ml, 1 h). Calnexin is shown as a loading control. (B) IκBβ and (C) ET-1 gene expression in RAW 264.7 pretreated with BAY-7085 (5–20 mmol/l, 1 h) or parthenolide (5–20 mmol/l, 1 h) before LPS exposure (1 mg/ml, 1 h). Values are means ± SEM (n = 4/time point). (D) Fold change in ET-1 protein secretion from RAW 264.7 cells pretreated with BAY-7085 (10 mmol/l, 1 h) or parthenolide (10 mmol/l, 1 h) before LPS exposure (1 mg/ml, 24 h) measured in cell medium by ELISA. Values are means ± SEM (n = 4/time point); *p < 0.05 versus unexposed control, †p < 0.05 versus LPS exposed.
Role of macrophage ET1 expression in the pathogenesis of persistent pulmonary hypertension of the newborn (continued)

References


The role of sirtuins and lysine acetylation in PAH

Background
Mounting evidence has implicated reprogramming of molecular metabolism in multiple cell and tissue sites as a key pathogenic mechanism in pulmonary arterial hypertension. The details of metabolic reprogramming have some cell type specificity, but in general tend to converge on glucose routing away from oxidation and toward lactate production with a concomitant shift toward reliance on specific alternative carbon sources (e.g., glutamine, fatty acids, other amino acids). Such metabolic reprogramming is reminiscent of the altered metabolic strategies exhibited by many malignancies. Understanding the molecular events that orchestrate these metabolic shifts are critical for identifying novel intervention strategies that target metabolism.

Of the many signaling pathways known to influence and coordinate cellular metabolic behavior, the sirtuin pathway is particularly situated to affect most or all of the metabolic pathways implicated in PAH pathogenesis. Sirtuins are in the superfamily of lysine deacetylases, with Sirt3 being the major mitochondrial isoform. Sirt3 is NAD+-dependent and redox regulated, positioning it optimally to be involved in the (dys)regulation of metabolism, oxidative stress, and mitochondrial function in PAH. Specific molecular targets previously implicated in PAH pathogenesis are known to be regulated by Sirt3, such as the mitochondrial isoform of superoxide dismutase (SOD2), lending further support to the idea that loss of Sirt3 activity may contribute to the development and maintenance of the PAH phenotype, and that targeting this pathway for intervention may be beneficial.

We hypothesized that loss of Sirt3 activity is a key pathogenic event in PAH, and that restoration of normal Sirt3 would ameliorate the molecular and pathophysiologic features of the PAH phenotype. We proposed to test this hypothesis using cell and mouse models in which PAH phenotype is driven by expression of a disease-causing mutation in bone morphogenic protein receptor type 2 (BMPR2).

Aims
We initially proposed the following 3 specific aims:
1. Test the hypothesis that lysine acetylation is increased and that sirtuin activity is decreased in the context of BMPR2-mediated PAH.
2. Test the hypothesis that decreasing sirtuin activity worsens the metabolic and cardiovascular phenotypes downstream from BMPR2 mutations, and that increasing sirtuin activity can rescue the PAH phenotype.
3. Test the hypothesis that sirtuin activity is decreased in patients with PAH.

Results
Our previous investigations of the altered metabolic landscape in BMPR2 mutant pulmonary microvascular endothelial cells (PMVEC) using snapshot metabolomics had shown a shift in carbon source utilization toward a reliance on glutamine as a carbon source to fuel the TCA cycle. We were able to further characterize this using stable isotope labeling strategies and confirmed that, in BMPR2-mediated PAH, the pulmonary endothelium become dependent upon glutamine. Glutamine is taken up more rapidly and is shuttled into the TCA cycle to a much greater extent in BMPR2 mutant PMVEC than in wild-type cells. BMPR2 mutant PMVEC are actually glutamine addicted, as limitations of glutamine tolerated by WT cells result in profound cell death in BMPR2 mutant PMVEC. We found that increased glutamine metabolism is
The role of sirtuins and lysine acetylation in PAH (continued)

dependent upon activation of hypoxia-inducible factor (HIF) in BMPR2 mutant PMVEC. Knowing that HIF activation lies downstream from loss of Sirt3 activity in some cancer types, and knowing that profound oxidant injury is present globally and specifically in the mitochondrial compartment in BMPR2 mutants, we hypothesized that Sirt3 was undergoing inactivation through deleterious covalent interactions with oxidized lipids in the mitochondrial membrane. We found that in BMPR2 mutant mitochondria, the Sirt3 content is the same as WT, but total lysine acetylation of the mitochondrial proteome is significantly increased, indicative of loss of Sirt3 function. We demonstrated that a specific class of oxidized lipids known to be increased in BMPR2-mediate PAH can inactivate Sirt3, and we then showed that a scavenger of these oxidized lipids (2-hydroxybenzylamine) would restore normal mitochondrial proteome acetylation and would prevent the development of PAH in BMPR2 mutant mice (Figure 1). Finally, we were able to demonstrate that the glutamine avidity present in cell and animal models is present in humans with PAH, as transpulmonary glutamine uptake measured at the time of right heart catheterization is significantly increased in PAH patients compared to individuals with normal hemodynamics and to patients with WHO Group III PH (Figure 2). These findings are summarized in a manuscript that is currently under review.

In addition to the above work, the lines of inquiry supported by the ENTELLIGENCE Young Investigator program have led to contributions to a number of other research projects, including investigations of dysregulated fatty acid metabolism and estrogen-dependent mechanisms of endothelial metabolic regulation in PAH, as well as translational investigations to develop better methods to assess small airway/small vessel diseases in humans.

Conclusion

Metabolic reprogramming is an important pathogenic mechanism in the endothelial compartment in PAH. With loss of BMPR2 function, severe oxidant injury in the mitochondria causes inactivation of Sirt3, leading to a glutamine-addicted phenotype. Targeting this mechanism with a molecular scavenger of damaging lipid peroxidation products restores Sirt3 activity and prevents development of PAH. Similar mechanisms appear to be at work in humans with PAH, as the in situ pulmonary vasculature exhibits a glutamine-avid phenotype.

We have identified a compound that can be used to target the Sirt3 pathway in humans, and the tools available to target specific metabolic pathways are improving daily. Directed modification of the metabolic network in specific cells and tissues is likely to be an important piece of the future treatment of pulmonary vascular disease.

The role of sirtuins and lysine acetylation in PAH (continued)

**Figure 1.** Panel A shows densitometry from Western blot analysis of total lysine acetylation in the mitochondrial proteomes of wild-type and Bmpr2 mutant mice treated with either vehicle or oral administration of 2-hydroxybenzylamine. Treatment with 2HOBA preserves Sirt3 activity, as evidenced by normalization of lysine acetylation in the treated Bmpr2 mutants. Panel B shows that in these same experimental groups, treatment with 2HOBA prevents the development of PAH in Bmpr2 mutant mice, as measured by total pulmonary resistance.

**Figure 2** shows transpulmonary glutamine gradients measured at the time of right heart catheterization in 3 groups of patients – WHO Group I PH (PAH), WHO Group III PH, and individuals with normal pulmonary hemodynamics. As predicted by in vitro and animal studies, PAH patients exhibit remarkable uptake of glutamine in the pulmonary vasculature, which is not seen in WHO Group III PH or in control individuals.
Background
Pulmonary hypertension (PH) is a complex disease with high morbidity and mortality and only modestly effective therapy. Chronic alveolar hypoxia associated with advanced lung diseases is a common cause of PH and associated right ventricular hypertrophy (RVH). Hypoxia’s impact on pulmonary vasculature is mediated through: (1) persistent vasoconstriction; and (2) vascular remodeling. Although the exact molecular mechanisms by which hypoxia exerts these effects are not well understood, there is increasing evidence that decreased nitric oxide (NO) bioactivity and aberrant NO signaling pathways are major factors involved in the pathogenesis of hypoxia-induced PH.

NO-based signaling classically involves the binding of NO to hemes in soluble guanylate cyclase (sGC) to increase cyclic guanosine monophosphate (cGMP). This pathway explains how NO bioactivity derived from the endothelium produces vascular relaxation. Besides heme, NO can covalently modify protein thiols, and it is increasingly apparent that the majority of NO’s functions in cellular signaling are carried out by S-nitrosylation, the covalent modification of cysteine thiols to form S-nitrosothiols (SNOs).

S-nitrosohemoglobin (SNO-Hb) is the prototypic S-nitrosylated protein that carries NO bioactivity in the circulation and can deploy it upon exposure to low oxygen tension, causing the physiologic response known as hypoxic vasodilation. This vasodilatory effect of SNOs in turn will increase the local blood flow and O2 delivery to match metabolic demand.

In addition to its systemic effect, NO bioactivity plays an important role in maintaining pulmonary vascular tone under normoxic condition and modulating hypoxic pulmonary vasoconstriction (HPV).

Impaired systemic hypoxic vasodilation and augmented HPV associated with decreased NO bioactivity can explain pathophysiologic changes in the pulmonary and systemic circulation seen in hypoxia-induced PH. In fact, numerous studies have shown that prolonged hypoxia associated with chronic lung diseases or high altitudes leads to depletion of NO bioactivity. Additionally, increased endogenous NO production can offset the pathophysiologic changes associated with chronic hypoxia, as seen in Tibetans living at high altitudes.

The current available therapies targeting NO-signaling pathways have only shown limited efficacy in treatment of hypoxia-induced PH. This can partially be explained by the fact that these medications act mainly on the sGC pathway within the pulmonary vasculature, and as such have limited ability to increase SNO-proteins and correct all the different aspects of NO-based signaling that are disrupted in hypoxia-induced PH. At the same time, the current state of knowledge on PH pathogenesis strongly supports the concept that administration of an S-nitrosylating agent to replete or maintain SNO homeostasis could have significant therapeutic potential. Collectively, these findings support the postulate that long-term nitrosylation therapy has the potential to correct SNO deficits (especially deficits in SNO-Hb) produced by hypoxia-induced PH, to improve systemic oxygenation and overall physiologic status.
S-Nitrosylation therapy to treat hypoxia-induced pulmonary arterial hypertension (continued)

Aims
1. To fully characterize the beneficial effects of an exogenous S-nitrosylating agent to correct or prevent pulmonary vascular and right ventricular changes induced by chronic hypoxia.
2. To determine the SNO-protein signature of chronic hypoxia and to identify the target proteins through which S-nitrosylation therapy exerts its effects.

Results
1. S-nitrosylating agent maintains SNO-Hb levels after exposure to chronic hypoxia. Exposure to chronic hypoxia is associated with a significant decline in RBC NO content, mainly in the form of reduced levels of SNO-Hb. In contrast, administration of S-nitrosylating agent yields levels of SNO-Hb that are significantly higher than that found in both the hypoxic and normoxic mice. This finding clearly demonstrates that chronic S-nitrosylation therapy can maintain SNO-Hb for prolonged periods (Figure 1).
2. S-nitrosylating agent attenuates and reverses hypoxia-induced RVH. Our data show that S-nitrosylating agent can significantly reduce hypoxia-induced RVH as reflected by reductions in Fulton’s Index (FI). These reductions are markedly greater than the benefit seen with sildenafil therapy, suggesting that targeting cGMP-independent SNO/NO pathways may be a superior therapeutic intervention for preventing the pulmonary vascular changes and RVH in response to a reduction in oxygen availability. Furthermore, in our therapeutic arm, treatment with S-nitrosylating agent under hypoxia condition reversed the RVH in mice exposed to hypoxia for two weeks prior to initiation of treatment (Figure 2).
3. S-nitrosylating agent therapy did not attenuate hypoxia-induced right ventricular systolic pressure (RVSP). Exposure to 14 days of chronic hypoxia resulted in marked increase in RVSP and pulmonary artery pressures. Concurrent treatment with S-nitrosylating agent tended to blunt this effect; however, the difference did not reach statistical significance.
4. Neither chronic hypoxia nor S-nitrosylating agent therapy resulted in a significant change in SNO-protein profile of the lungs or heart tissue. We assessed the SNO-protein proteome of heart and lungs using SNO-RAC and Coomassie staining. Samples from normoxia, hypoxia and S-nitrosylating agent treated mice were used. The results for the heart and lungs showed no difference between the samples from the three groups.

Conclusion
Our study showed that inhalation of S-nitrosylating agent during exposure to chronic hypoxia can prevent hypoxia-induced pulmonary hypertensive changes, as demonstrated by attenuation of the RVH. Furthermore, S-nitrosylating agent can also have a therapeutic benefit in established PH by preventing further progress and/or reversal of RVH despite continuous exposure to hypoxia. Given RV hypertrophy and failure is the major cause of morbidity and mortality associated with PH, these findings can potentially have significant clinical impact.

In terms of the mechanisms by which S-nitrosylating agent can prevent and reverse RVH, our studies could not clearly discover a mechanism. Based on established literature, we postulated that by increasing levels of SNO-Hb and therefore NO bioactivity, S-nitrosylating agent could attenuate HPV and thereby prevent RVH. However, right heart pressure measurements in S-nitrosylating agent treated versus hypoxia alone mice did not support our theory. Next we hypothesized that S-nitrosylating agent modulates its effect on RV directly by changing the SNO-protein profile of the cardiomyocytes. Again, our limited data do not support this theory, since chronic hypoxia was not associated with a marked reduction in SNO-proteins and the SNO-protein profile of S-nitrosylating agent treated mice was very similar to both normoxic and hypoxic mice. It is, however, possible that NO bioactivity conveyed through SNO-Hb can alter the expression of different proteins in the heart tissue and thereby attenuate or reverse the hypoxia-induced RVH. Hence, assessment of the total protein proteomes of the heart tissue is worth investigating.
S-Nitrosylation therapy to treat hypoxia-induced pulmonary arterial hypertension (continued)

Figure 1. Chronic hypoxia led to depletion in SNO-Hb. Treatment with S-nitrosylating agent (SNA) resulted in significantly higher SNO-Hb levels when compared to normoxia and hypoxia groups (p<0.05).

![Graph showing NO concentration in various conditions](image1)

Figure 2. Chronic hypoxia induces right ventricular hypertrophy and results in increased Fulton Index. Administration of S-nitrosylating agent (SNA) during exposure to hypoxia can attenuate and reverse these changes. *p<0.05 vs 4WH+2WS.

4WH: Hypoxia for 4 weeks.
4WH + 2WS: 2 weeks of hypoxia followed by administration of S-nitrosylating agent while exposed to hypoxia for 2 more weeks.
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Biographies

Ronald J. Oudiz, MD
ENTEILLIGENCE Steering Committee Chair
Director, Liu Center for Pulmonary Hypertension
LA Biomedical Research Institute at Harbor-UCLA Medical Center
Professor of Medicine
The David Geffen School of Medicine at UCLA
Torrance, CA

Dr. Oudiz is Professor of Medicine, David Geffen School of Medicine at UCLA and is the Director of the Pulmonary Hypertension Center and Faculty Cardiologist at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center in Southern California. He received his medical school training at the University of Southern California in Los Angeles, his Internal Medicine training at the University of California, San Diego, and his training in Cardiovascular Diseases at Harbor-UCLA Medical Center in Torrance, CA. He is board-certified in Internal Medicine and Cardiovascular Diseases. Dr. Oudiz is a past holder of scientific research awards from the American Heart Association and the National Institutes of Health. He has authored several papers in pulmonary hypertension and has presented his research at national and international seminars. Dr. Oudiz is the past Editor-in-Chief of the scientific publication Advances in Pulmonary Hypertension. He has participated in several trials of innovative medical treatments for pulmonary hypertension (PH), many of which are still ongoing. Dr. Oudiz's recent focus has been to describe the physiologic abnormalities that are caused by PH using measurements of lung gas exchange during exercise, and to study exercise rehabilitation as a treatment modality for patients with PH.

Harrison W. Farber, MD
Professor of Medicine
Boston University School of Medicine
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Boston Medical Center
Boston, MA

Dr. Farber is a Professor in the Department of Medicine and the Director of the Pulmonary Hypertension Center at Boston University. He has focused on research into pulmonary arterial hypertension (PAH) and the clinical care of PAH patients for approximately 20 years. Dr. Farber has received numerous grants (both basic science and clinical) and has an extensive publication record in this area, including articles in peer reviewed journals such as Circulation, New England Journal of Medicine, and Chest. He serves on many panels for the development of clinical recommendations in PAH, has participated in large multicenter clinical trials, and was on the Steering Committee of the REVEAL Registry (Registry to Evaluate Early and Long Term PAH Disease Management), the largest registry of PAH patients ever created. His research interests include endothelial cell biology, in particular, the response of the pulmonary vasculature to injury. After earning a medical degree at George Washington University School of Medicine, Dr. Farber completed an internship and residency at the Medical College of Virginia and a fellowship at Boston University.
Biographies

Mardi Gomberg-Maitland, MD, MSc
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Maureen D. Mayes, MD, MPH
Professor of Internal Medicine
Elizabeth Bidgood Chair in Rheumatology
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Dr. Gomberg-Maitland is Associate Professor of Medicine and Director of the Pulmonary Hypertension Program at the University of Chicago Medical Center in Chicago, IL. She earned her undergraduate degree at Yale University, her medical degree at the Albert Einstein College of Medicine and completed a residency at New York Presbyterian Hospital-Weill/Cornell Medical Center and a fellowship at Mount Sinai Medical Center. She earned a Masters in Clinical Epidemiology at Harvard School of Public Health. Dr. Gomberg-Maitland is an expert clinician and researcher in the field of pulmonary heart disease. In recent years, she has participated in dozens of multicenter, multinational research trials to explore new therapies for pulmonary hypertension. She is currently focusing on pulmonary arterial, pulmonary venous hypertension/ diastolic dysfunction, and biomarker development. A fellow of the American College of Cardiology, American College of Chest Physicians, and American Heart Association, and a member of the International Society of Heart and Lung Transplantation, American Thoracic Society, and Pulmonary Hypertension Association, she has published numerous articles in peer-reviewed journals, including Circulation, Journal of the American College of Cardiology, Clinical Pharmacology and Therapeutics, Chest, European Respiratory Journal, and the American Journal of Respiratory and Critical Care Medicine.

Dr. Mayes graduated from Eastern Virginia Medical School and completed her Internal Medicine training and Rheumatology fellowship at the Cleveland Clinic. She received a Master’s in Public Health (MPH) in Epidemiology from the University of Michigan School of Public Health. She joined the University of Texas – Houston Medical School faculty in 2002 and subsequently established the Scleroderma Clinic. Dr. Mayes is the recipient of many distinctions, awards and grants for the study and treatment of scleroderma. She is the author of over 180 published manuscripts, 26 reviews, 9 book chapters and 2 full length books. Her clinical interests include the treatment of scleroderma and its multiple complications. She participates in several multi-center, national trials of new agents for this disease. Her research interests include the identification of susceptibility genes and disease severity genes in scleroderma and related autoimmune diseases. She is currently the Principal Investigator of the NIH/NIAMS funded ‘Genome-Wide Association Study in Systemic Sclerosis’ that has the objective of identifying genes that influence disease susceptibility and severity; and PI of the Scleroderma Family Registry and DNA Repository, which serves as a national resource to supply genetic material to other investigators to study this disease.
Dr. Michelakis was born in Greece, where he went to Medical School at the University of Patras. He completed training in Vascular Biology, Internal Medicine, and Cardiology at the University of Texas (Galveston), Yale University, and the University of Minnesota. He joined the faculty of the University of Alberta in 1998, where he is now a full Professor and a Vice Chair (Research) in the Department of Medicine. Dr. Michelakis founded and has directed the Pulmonary Hypertension Program and clinic at the University of Alberta since 2001; this multidisciplinary clinic is open 5 days a week and treats patients referred from Alberta, Northern BC, Saskatchewan and Manitoba. He is also a vascular biologist and runs an active laboratory with several graduate students and technicians, focusing on the discovery of novel therapies for pulmonary hypertension. He is the Canada Research Chair in Applied Molecular and Mitochondrial Medicine and the Chair of the Cardiopulmonary, Critical Care, Perioperative and Resuscitation (3CPR) Council of the American Heart Association, and he serves on the editorial boards of both Circulation and Circulation Research. Dr. Michelakis has discovered intriguing similarities in the biology of pulmonary hypertension and cancer, which have led him into an exciting translational research program in cancer as well.

Dr. Palevsky is a Professor of Medicine at the Perelman School of Medicine of the University of Pennsylvania. He is also Chief of the Pulmonary, Allergy and Critical Care Division and Director of the Pulmonary Vascular Disease Program at the Penn Presbyterian Medical Center in Philadelphia. The Pulmonary Vascular Disease Program is a multi-disciplinary program focusing on the diagnosis and treatment of pulmonary vascular disease, pulmonary arterial hypertension, and pulmonary thromboembolic disease, both acute and chronic. Dr. Palevsky earned a medical degree from the Medical College of Virginia. He completed an internship and residency in internal medicine, and a fellowship in pulmonary and critical care medicine at the Hospital of the University of Pennsylvania, where he worked with Alfred P. Fishman, MD. His clinical and research interests include unexplained dyspnea, pulmonary vascular disease, pulmonary hypertension, and thromboembolic disease. Dr. Palevsky has been published in numerous peer-reviewed journals, including the Annals of Internal Medicine, JAMA, and Circulation. He has been recognized as one of Philadelphia’s “Top Docs” and is included in national lists such as “The Best Doctors in America” and the “Guide to America’s Top Physicians.”
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Biographies

Richard M. Silver, MD
Distinguished University Professor of Medicine and Pediatrics
Director, Division of Rheumatology & Immunology
Medical University of South Carolina
Charleston, SC

Dr. Silver serves as Director of the Division of Rheumatology & Immunology at the Medical University of South Carolina (MUSC). He is a graduate of the University of Tennessee–Knoxville and earned his MD from Vanderbilt University School of Medicine. Dr. Silver completed training in Internal Medicine at the University of North Carolina-Chapel Hill and in Rheumatology at London’s Northwick Park Hospital and at the University of California-San Diego. He joined the faculty at the Medical University of South Carolina in 1981, and has served as Director of the Division of Rheumatology & Immunology since 1995. MUSC’s Board of Trustees named him a “Master Teacher” and bestowed the University’s highest academic recognition, Distinguished University Professor. The Scleroderma Foundation named him their “Doctor of the Year” in 2007. Dr. Silver’s major research interest is interstitial lung disease associated with systemic sclerosis.

Kurt R. Stenmark, MD
Professor of Pediatrics, Medicine, and Anesthesiology
Division Head, Pediatric Critical Care Medicine
Director, Cardiovascular Pulmonary Research Laboratory
University of Colorado Anschutz Medical Campus
Aurora, CO

Dr. Stenmark is a Professor of Pediatrics, Medicine, and Anesthesiology at the University of Colorado Anschutz Medical Campus in Aurora, CO. He is also the Division Head of Pediatric Critical Care Medicine and Director of the Cardiovascular Pulmonary Research Laboratory. Dr. Stenmark earned his medical degree at the University of Colorado, Denver and completed his internship and residency at the University of Colorado Health Sciences Center (UCHSC), where he was the Pediatric Chief Resident. He completed a Pediatric Critical Care fellowship at The Children’s Hospital and a Cardiovascular Pulmonary Research fellowship at UCHSC. Dr. Stenmark’s clinical and research interests include cellular and molecular mechanisms that contribute to structural remodeling of the pulmonary vasculature and to right heart dysfunction in the setting of pulmonary hypertension. He has served as an Associate Editor for the American Journal of Physiology - Lung Cellular and Molecular Physiology, and on the editorial boards of several journals, including American Review of Respiratory and Critical Care Medicine, Circulation Research, and Pulmonary Circulation. He is a member of the ATS Scientific Advisory Council, Scleroderma Foundation PeerReview, and Pulmonary Vascular Research Institute (PVRI) Steering and Scientific committees. A featured speaker at numerous conferences, Dr. Stenmark has published book chapters and over 289 manuscripts in peer-reviewed journals, including, but not limited to, the New England Journal of Medicine, Science, Journal of Clinical Investigation, Circulation Research, and the American Journal of Physiology.
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Biographies

Jason X-J Yuan, MD, PhD
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Dr. Yuan is Professor of Medicine and Associate Vice President for Translational Health Sciences at the University of Arizona in Tucson, AZ. He is also Director of the Division of Translational and Regenerative Medicine in the Department of Medicine at the University Of Arizona College Of Medicine. Dr. Yuan received his medical school training at Suzhou Medical College (China), his PhD at Peking Union Medical College (China), and his postdoctoral training at the University Of Maryland School Of Medicine. His pulmonary vascular disease research propels the field on pathogenic roles of membrane receptors and ion channels and provides a new research direction for developing therapeutic approaches for the disease. Dr. Yuan is a Fellow of the American Heart Association, the American Association for the Advancement of Science, and the American Physiological Society. He is an elected Member of the American Society for Clinical Investigation and the Association of American Physicians. Dr. Yuan has served on many advisory committees and editorial boards, including Chair of the Respiratory Integrative Biology and Translational Research study section of the National Institutes of Health. He is currently Chair of the Pulmonary Circulation Assembly of the American Thoracic Society, Editor-in-Chief of the journal Pulmonary Circulation, and Associate Editor of the American Journal of Physiology - Cell Physiology.