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

Ronald J. Oudiz, MD
Chairman of the ENTELLIGENCE Steering Committee

Dear Colleagues,

We are delighted to announce that in 2020, the ENTELLIGENCE™ Young Investigator Program chose four new young investigators to receive ENTELLIGENCE awards based on their outstanding pulmonary vascular disease-related research proposals. This year, we received extremely high quality, competitive submissions, and we are proud to recognize these four young leaders. 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 15 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 71 promising researchers in the field of pulmonary vascular disease have been awarded to date.

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 50 peer-reviewed journals, including American Journal of Physiology, American Journal of Respiratory and Critical Care Medicine, Chest, Circulation, Nature, 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.

Best regards,

Ronald J. Oudiz, MD

Tentative

ENTEILLIGENCE Young Investigator Program Timeline

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<th>2020</th>
<th>Sep</th>
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<td>Grant Review Feb. 9 – Feb. 23</td>
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<td>Letter of Intent (LOI) Submission July 20 – Nov. 2</td>
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<td>LOI Selection Conference Call Dec. 9</td>
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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.entiligencemd.org.

Review cycles completed: 15
Awards distributed: 71
Overview of ENTELLIGENCE Awards

Awarded 2020

Arun Jose, MD
University of Cincinnati
Cincinnati, OH
Co-Investigator: William C. Nichols, PhD
Mentors: Jean M. Elwing, MD, and Francis X. McCormack, MD
Project Title: Biomarker Discovery in Portopulmonary Hypertension

Claudia Mickael, PhD
University of Colorado, Denver
Department of Medicine
Division of Pulmonary Sciences and Critical Care Medicine
Aurora, CO
Mentor: Rubin Tuder, MD
Project Title: The Role of Classical Dendritic Cells in Pulmonary Hypertension

Maryam Sharifi-Sanjani, PhD
University of Pittsburgh
Pittsburgh Heart, Lung and Blood Vascular Medicine Institute
Division of Cardiology
Pittsburgh, PA
Co-Investigator: Dennis Bruemmer, MD, PhD
Mentors: Stephen Chan, MD, PhD, and Imad Al Ghouleh, PhD
Project Title: Insight into Right Ventricular Defenses to Pulmonary Arterial Hypertension: Potential Role of TRF2, A Telomeric Protein

Kel Vin Woo, MD, PhD
Washington University
Department of Pediatrics, Division of Cardiology
St. Louis, MO
Mentors: David Curiel, MD, PhD, and David Ornitz, MD, PhD
Project Title: Endothelial Drug Targets for Hypoxia-Induced Pulmonary Hypertension
Overview of ENTELLIGENCE Awards

2020 Award Winners

From left: Arun Jose, MD; Claudia Mickael, PhD; Maryam Sharifi-Sanjani, PhD; and Kel Vin Woo, MD
Overview of ENTELLIGENCE Awards

Awarded 2019

Mark Dodson, MD, PhD
Intermountain Medical Center
Murray, UT
Mentors: Gregory Elliott, MD, and Lisa Cannon-Albright, PhD
Project Title: Identifying Genetic Risk Factors for Chronic Thromboembolic Pulmonary Hypertension

Jonathan Edwards, MD
Children’s Hospital of Philadelphia
Philadelphia, PA
Mentors: Zoltan Arany, MD, and Laura Mercer-Rosa, MD
Project Title: Investigating Altered Right Ventricular Myocardial Gene Expression and Biomarkers Patterns Associated with Adaptive and Maladaptive Remodeling in Diverse Human Models

Andrea Frump, PhD
Indiana University School of Medicine
Indianapolis, IN
Co-Investigator: Mark Geraci, MD
Mentors: Tim Lahm, MD, and Micheala Aldred, PhD
Project Title: Identification of the Apelin-mediated Transcriptome in Right Ventricular Failure using Single Cell RNA-Seq

Victor Tseng, MD
Emory University
Atlanta, GA
Mentors: C. Michael Hart, MD, and Eva Grayck, MD
Project Title: Hyaluronan Drives Pathologic Vascular Metabolism in Pulmonary Hypertension
Overview of ENTELLIGENCE Awards

2019 Award Winners

From left: Mark Dodson, MD, PhD; Jonathan Edwards, MD; Victor Tseng, MD; and Andrea Frump, PhD
Overview of ENTELLIGENCE Awards

Awarded 2018

Catherine Avitabile, MD  
University of Pennsylvania Perelman School of Medicine  
Philadelphia, PA  
**Mentors:** Stephen Paridon, MD, and Babette Zemel, PhD  
**Project Title:** The association between skeletal muscle deficits and exercise performance in pediatric pulmonary hypertension patients

Meghan Bernier, MD  
The Johns Hopkins University School of Medicine  
Baltimore, MD  
**Co-Investigator:** Larissa Shimoda, PhD  
**Mentor:** Lewis Romer, MD  
**Project Title:** Endothelial to mesenchymal transition in pulmonary hypertension: Formin a new identity

Stephen J. Coleman, MS, PhD  
Colorado State University  
Fort Collins, CO  
**Mentor:** Milton Thomas, PhD  
**Project Title:** Investigation of calcium regulatory processes and their role in a natural large animal model of altitude-associated pulmonary hypertension sensitivity leading to heart failure

Daniel Lachant, DO  
University of Rochester Medical Center  
Rochester, NY  
**Mentor:** R. James White, MD  
**Project Title:** Extracellular vesicles as a marker of vascular disease activity in PAH
Overview of ENTELLIGENCE Awards

Awarded 2017

Nadine Al-Naamani, MD, MS
University of Pennsylvania
Philadelphia, PA
Co-Investigator: David Lederer, MD
Mentor: Steven Kawut, MD
Project Title: Exploring the association of visceral intrathoracic fat with vascular stiffness in pulmonary hypertension
• Presented at 2018 and 2019 American Thoracic Society Conference

Jonathan Davies, MD
Baylor College of Medicine
Houston, TX
Mentor: Michael Blackburn, PhD
Project Title: The role of adenosine signaling in pulmonary hypertension associated with bronchopulmonary dysplasia

Rebecca Kameny, MD
University of California, San Francisco
San Francisco, CA
Mentor: Jeffrey Fineman, MD
Project Title: Translating the natural history of pulmonary vascular disease secondary to congenital heart disease into basic mechanisms and therapeutic targets
• Presented at 2018 PVRI World Congress and 2018 Pediatric Academic Societies Meeting
• Published in American Journal of Respiratory Cell and Molecular Biology, 2019

Stephanie Thorn, PhD
Yale University
New Haven, CT
Mentors: Hyung Chun, MD, and Albert Sinusas, MD
Project Title: Pilot study to engage the Apelin-MEF2 signaling axis for myocardial preservation in a large animal model of right ventricle failure
• Presented at American Society of Nuclear Cardiology, 2018
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
- Presented at 2017 American Thoracic International Conference; 2017 PVRI World Congress; and 2016 American Heart Association Scientific Sessions
- Published in American Journal of Respiratory and Critical Care Medicine, 2018

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

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
- Presented at 2018 American Thoracic Society Conference and 2017 American Heart Association Scientific Sessions
- Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2018 and British Journal of Pharmacology, 2017

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
- Presented at 2017 PVRI World Congress

Project Title: Impact of mitochondrial heat shock protein 90 inhibition in pulmonary arterial hypertension
- Presented at 2017 American Thoracic International Conference; 2017 PVRI World Congress; and 2016 American Heart Association Scientific Sessions
- Published in American Journal of Respiratory and Critical Care Medicine, 2018
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


• Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2015, 2016 and 2017; Canadian Journal of Cardiology, 2015 and 2016; and Circulation, 2015 and 2016

Marshaleen N. Henriques King, MD, MSc
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

• Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2016

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: MATCH-uPP - MRI and catheterization hemodynamics in pediatric pulmonary hypertension

• Presented at 2015 and 2016 American Heart Association Scientific Sessions; 2016 Society of Cardiac Magnetic Resonance Scientific Sessions; 2016 American Thoracic Society Conference; and 2016 UCSF Pulmonary Hypertension Conference

• Published in European Heart Journal - Cardiovascular Imaging, 2017 and 2018; Pediatric Cardiology, 2018; The Journal of Heart and Lung Transplantation, 2018; Circulation: Cardiovascular Imaging, 2017; and Oxidative Medicine and Cellular Longevity, 2017
**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  

**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  
- Presented at 2015 American Thoracic Society Conference and 2015 International Society for Heart & Lung Transplantation  
- Published abstracts: Journal of Heart and Lung Transplantation, 2015 and American Journal of Respiratory and Critical Care Medicine, 2015

**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 Meeting  
- 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 (PPHN) caused by perinatal inflammation  
- 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 Conference; 2015 European Respiratory Society Congress; and 2014 Keystone Symposium on Molecular and Cellular Biology

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. Shinohara, 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, 2014; 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**  
- Published in Pulmonary Circulation, 2017 and 2018; Theranostics, 2018; Scientific Reports, 2016; Chest, 2015; PLoS One, 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  
- Published in Pulmonary Circulation, 2016 and 2017
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 Scientific Sessions

Marco Mura, MD, PhD
University of Toronto
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 Respirology, 2019; 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 American Journal of Pathology, 2013

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 Meeting
• Published in American Journal of Physiology - Lung Cellular and Molecular Physiology, 2013 and 2014; and Pediatric Research, 2013 and 2014

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 in Human Gene Therapy, 2017
• Published abstract: Molecular Therapy Supplement, 2012

Michael York, MD
Boston University Medical Center
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 ENTTELLIGENCE 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 Conference
• 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 Scientific Sessions and 2009 American Thoracic Society Conference
• Published in Circulation, 2015; Circulation Research, 2013; Nature Medicine, 2013; Arteriosclerosis, Thrombosis, and Vascular Biology, 2011; and Journal of Heart and Lung Transplantation, 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 European Respiratory Society Congress
• 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 Queen’s 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 Conference
  • 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

Awards 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 Journal of Molecular Medicine, 2014 and European Respiratory Journal, 2008

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 for Heart & 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
Introduction:
Pulmonary arterial hypertension (PAH) is a vascular disease characterized by progressive narrowing of the small pulmonary arteries. Portopulmonary hypertension (PoPH) is a type of PAH that occurs exclusively in patients with underlying liver disease, affecting up to 10% of all patients undergoing liver transplantation and exhibiting the highest morbidity and mortality of all PAH subtypes.

Background:
Liver sinusoidal endothelial cell (LSEC) and pericyte injury are believed to play a central role in disease pathogenesis, potentially through the release of inflammatory vasoactive mediators acting on the matrix metalloproteinase 9 (MMP-9) and bone morphogenetic protein 9 (BMP-9) pathways, but the specific gene expression profiles, potentially mechanistic upregulated biochemical processes, and corresponding biomarkers unique to these cells in PoPH patients have not yet been studied. The advent of single-nucleus RNA sequencing (snRNAseq) technology provides an unparalleled opportunity to study the genetic profile of specific PoPH cellular populations in their native niche with an unprecedented degree of detail, allowing for the identification of transcriptomic profiles, signaling pathways, and potential circulating biomarkers that may be responsible for driving PoPH disease pathogenesis. We are in a unique position to apply this breakthrough technology to the study of hepatic endothelial and stellate cells in PoPH, which to our knowledge has not yet been attempted. We have recently completed snRNAseq on liver tissue obtained intraoperatively from a 55-year-old female with PoPH and identified a number of differentially expressed genes known to be associated with PAH endothelial cells (including epidermal and platelet-derived growth factor, genes for the Ras and metalloproteinase signaling pathways, and endoglin, an inhibitor of BMP-9) in both LSEC and pericyte cell populations.

Hypothesis and Specific Aims:
We propose using snRNAseq to isolate and characterize gene expression patterns, signaling pathways, and receptor/ligand interactomes that are unique to PoPH and correspond to the MMP-9 and BMP-9 pathways; identify circulating biomarkers corresponding to these patterns; and validate the diagnostic and prognostic value of these biomarkers using the resources of the PAH Biobank.
**2020 Abstracts**

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University of Colorado, Denver  
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*The Role of Classical Dendritic Cells in Pulmonary Hypertension*

**Introduction:**  
Pulmonary hypertension (PH) is often fatal and is characterized by elevated right ventricle pressures and increased vascular remodeling and resistance.

**Background:**  
Dysregulated immunity underlies the pathophysiology of PH, which is demonstrated by elevated numbers of inflammatory cells around the remodeled vessels, as well as high levels of inflammatory cytokines present in the plasma of patients from different PH groups. Notably, there is a substantial body of evidence indicating that dendritic cells are orchestrators in this process; however, there are few studies that address the pathogenic mechanisms through which these cells could participate in PH.

**Hypothesis and Specific Aims:**  
Our goal is to determine the mechanistic role of dendritic cells driving inflammation in the context of hypoxia, which leads to vascular disease. This project will unravel pathophysiological mechanisms of classical dendritic cells (cDCs) in hypoxia-PH by using recombinant murine animals and single-cell RNA sequencing. We propose that the activation of these cells by a hypoxic stimulus can trigger secretion of cytokines, chemokines, and growth factors by the vascular bed, driving the remodeling and changing of the perivascular environment and potentially leading to recruitment of dendritic cells and macrophage/macrophage influx into the lung perivascular compartment. We have supportive preliminary data revealing that classical dendritic cells are pathogenic in hypoxia-induced PH, are increased in lungs of hypoxia-challenged mice, and are probably involved in monocyte/macrophage recruitment into the lung. This project will be highly innovative in pulmonary vascular diseases and will open an unexplored and rich investigative research field.
Introduction:
Right ventricular (RV) failure due to pressure overload is the leading cause of morbidity and mortality in pulmonary arterial hypertension (PAH). However, the underlying causes continue to be poorly understood, and identifying effective RV targeted therapy remains unresolved.

Background:
Preliminary data support the mitigated expression of telomeric repeat-binding factor 2 (TRF2), an essential telomere length-protecting protein, in RVs of PAH mouse models of hypoxia and pressure-overload model of pulmonary arterial banding (PAB), while its expression does not change in the lungs or cardiac fibroblasts. These data suggest that TRF2 is a cardiomyocyte (CM)-specific signal affected by PAH models. Therefore, the principal investigator (PI) generated a unique mouse with constitutive CM-specific deletion of TRF2 (cTRF2+/-). Preliminary data show premature RV dysfunction and early death in cTRF2+/- mice similar to late stages of PAH without concurrent CM telomere attrition, supportive of a telomere length maintenance-independent role for TRF2 in CMs. Interestingly, cTRF2+/- CMs present relocalized H3K9me3 (a marker of heterochromatin) away from the nuclear periphery, a hallmark of gene transcription activation, coupled with aberrant RV dysfunction-associated gene expression.

Hypothesis and Objectives:
The PI hypothesizes that TRF2 governs RV cardiomyocyte gene transcription changes associated with RV dysfunction in PAH via regulating nuclear chromatin localization.

Specific Aims:
To test this hypothesis, we will characterize RV function and morphology in tamoxifen-induced conditional CM-specific TRF2-deficient (conTRF2+/-) mice following hypoxia and PAB. To identify whether CM-TRF2 deficiency alters the expression of RV dysfunction-associated genes via chromatin relocalization, we will assess H3K9me3-DNA interaction at genomic binding sites of candidate genes by chromatin immunoprecipitation (ChIP)-qPCR. Further, nuclear relocalization (distance from nuclear periphery) of core histones and the above-identified activated genes will be evaluated via state-of-the-art high-resolution 2D FISH (fluorescence in situ hybridization) immunostaining. To determine whether TRF2 overexpression has translational therapeutic potential in humans, we will adenovirally overexpress TRF2 in human-induced pluripotent stem cell (hiPSC)-derived CMs with and without hypoxia or TNF-α (PAH-related stimuli) in the presence and absence of heterochromatin protein1 (known to induce chromatin compaction via H3K9me3) siRNA and outcome on the expression of PAH-associated RV dysfunction genes and nuclear localization evaluated.
Introduction:
Pulmonary hypertension (PH) is a debilitating disease characterized by pathologic changes of endothelial cell (EC) function, leading to increased pulmonary vascular resistance. Group 3 PH, caused by lung disease and/or hypoxia, including bronchopulmonary dysplasia (BPD) and chronic obstructive lung disease (COPD), is the second largest cause of mortality in PH patients. PH further increases the morbidity and mortality in both COPD and BPD patients. There is an unmet medical need to understand the mechanisms of pulmonary vascular remodeling in group 3 PH and to identify novel therapeutic targets.

Background:
Fibroblast growth factor (FGF) 2 and FGF receptor 1 (FGFR1) are elevated in the lung tissue of PH patients. We have developed a physiologically relevant hypoxia mouse model that mimics group 3 PH. We showed that the inactivation of FGFRs in ECs worsens PH, while the expression of a constitutively active FGFR1 (caFGFR1) in ECs protects against hypoxia-induced PH. These data suggest that FGF activity plays a vital role, signaling in part via endothelial FGFR1, to protect against group 3 PH.

Hypothesis and Specific Aims:
We hypothesize that direct activation of FGF signaling or FGFR-regulated signaling pathways in ECs will protect against PH. In this proposal, we will identify targets of EC-FGFR1 signaling involved in the protection of hypoxia-induced PH, and we will develop a viral vector to therapeutically activate FGF signaling in ECs.

Specific Aims:
To achieve these goals, we will identify associated FGF signaling pathways activated in hypoxia-challenged EC cultures, and we will use translating ribosome affinity purification technology to identify and characterize FGFR-regulated transcripts in ECs in vivo. We will develop an in vivo adenovirus (Ad) system to deliver activated FGFR1 to pulmonary ECs. We will use an engineered adenovirus (Ad5.MBP) with tropism for ECs to construct an Ad5.MBP.caFGFR1 delivery package and test its efficacy in preventing or reversing the pulmonary vascular remodeling in hypoxia-induced PH. This project will advance our understanding of PH and explore a potential therapeutic approach that could prevent or even reverse the effects of hypoxia on PH.
Introduction and Background:
Chronic thromboembolic pulmonary hypertension (CTEPH) is a debilitating condition caused by chronic obstruction of the pulmonary arteries by thromboembolic material arising from incomplete resolution of pulmonary embolism (PE). Existing data suggest that the majority of CTEPH cases go undiagnosed, and when a correct diagnosis of CTEPH is made there is often an unacceptably long diagnostic delay. There are two major reasons for this. First, CTEPH occurs in only a small percentage of PE survivors (approximately 3%), making screening of all PE survivors for subsequent development of CTEPH inefficient. Second, the mechanisms leading to incomplete thrombus resolution after acute PE are not well understood, and thus no clinically useful biomarkers of future risk of CTEPH have been identified.

The goal of this proposal is to identify genetic markers associated with CTEPH risk. These genetic markers could ultimately be integrated into a risk prediction model for CTEPH, which may improve case recognition by allowing for the triage of high-risk patients to more intensive surveillance for CTEPH after acute PE. In a recently published manuscript, our group has used the Utah Population Database (UPDB), a unique genealogical database that includes a majority of families in the state of Utah, to show that patients with CTEPH are significantly more related than would be expected by chance. These data provide the strongest evidence to date that genetic factors influence CTEPH risk. In this proposal, we aim to use unique Utah genetic resources to gain further insight into the types of genetic variants that are associated with CTEPH, and then use both candidate-based and unbiased screening methods to identify CTEPH predisposition genetic variants.

Hypothesis:
The unifying hypothesis of this proposal is that genetic factors influence CTEPH risk, and that these genetic factors are distinct from the common inherited thrombophilias that influence the risk of acute uncomplicated VTE. We hypothesize the existence of a set of genetic variants that specifically predispose to VTE events that do not resolve and thus predispose to CTEPH. This set of genetic variants contrasts with the common inherited thrombophilias (including the Factor V Leiden mutation), which we hypothesize predispose to VTE events that are likely to resolve and thus unlikely to lead to CTEPH.

Specific Aim 1:
Determine the frequency of the Factor V Leiden mutation in patients with CTEPH and in carefully matched patients with acute uncomplicated PE in whom CTEPH is not suspected.

Specific Aim 2:
Use the UPDB to determine whether CTEPH high risk pedigrees are also VTE high risk pedigrees, and whether probands from these pedigrees carry common inherited thrombophilias.

Specific Aim 3:
Use a powerful pedigree-based whole genome sequencing approach to identify and validate novel candidate genetic variants underlying CTEPH risk in Utah high risk CTEPH pedigrees.
Introduction and Background:
The mechanisms of adaptive remodeling and pathologic right ventricular (RV) remodeling, which lead to preserved RV function (pRV) or RV failure (RVF) respectively, are poorly understood despite RVF being the primary cause of hospitalization and mortality in PAH. The molecular mechanisms that drive adaptive and pathologic RV remodeling in PAH have remained incompletely characterized due to lack of tissue availability, particularly for pRV. However, given that previously implicated pathways in RV remodeling appear to be involved in multiple etiologies, we will use the availability of RV tissue from left ventricular failure (LVF) to our advantage. From more than 150 ischemic (ICM) and 200 dilated cardiomyopathy (DCM) patients in the Penn Human Heart Tissue Bank, we will identify pRV and RVF using preexplant hemodynamics and compare to nonfailing (NF) controls as a discovery cohort. We will leverage ongoing bulk RNAseq to identify representative age, gender, and ethnicity-matched DCM-pRV, DCM-RVF, ICM-pRV, and ICM-RVF samples to assess using a single nuclear RNAseq method sNucDrop-seq. sNucDropseq will provide unique insight into cell population source for differential gene expression, the role of cardiomyocyte and noncardiomyocyte heterogeneity in adaptive and pathologic remodeling, and uncover the role of noncardiomyocyte cell populations that can be averaged out at the bulk RNAseq level.

Next, novel RV remodeling targets will be tested against clinically used and recently identified biomarkers of ventricular remodeling (NT-proBNP, MMP1, MMP9, and GAL3) using echocardiographic indices of RV remodeling and function in two pediatric cohorts. The first will be comprised of Nice classification group 1 PAH serum and the second will be comprised of serial tetralogy of Fallot (TOF) preoperative serum, RV tissue collected at surgery, and serum at one year follow up.

Hypothesis:
Through these studies, we hope to identify novel prognostic and therapeutic targets of RV remodeling, which will have broad applicability across multiple etiologies and demographics groups, and will ultimately lead to improved clinical outcomes for all patients with RVF.

Specific Aim 1:
Determine the cell-specific transcriptional signature of RV myocardium in pRV and RVF in the setting of LVF as a discovery cohort.

Specific Aim 2:
Test novel, cardiomyocyte and non-cardiomyocyte RV remodeling expression targets and biomarkers in pediatric PAH and TOF.
Introduction and Background:
RV function is the primary prognostic factor for both morbidity and mortality in PH, but despite this importance, no RV-directed therapies exist. Furthermore, patients with more common cardiopulmonary diseases such as chronic obstructive pulmonary disease, pulmonary fibrosis, sleep disordered breathing and left heart disease are at major risk for developing PH and/or RV failure. Intriguingly, despite increased susceptibility to PH, women have better RV function and survive longer with PH than men. Therefore, there is an unmet, scientific need to identify the sex- and cell-specific pathways driving RV failure, and to target those pathways to prevent, delay or reverse RV failure. We hypothesize that apelin-mediated signaling will promote a prolonged adaptive remodeling response in the pressure-overloaded RV. The goal of this proposal is to identify the hitherto unknown molecular, sex and cell-specific targets of apelin during the progression of RV failure and how it may be used to prevent or delay RV failure, which would allow for the development of novel, RV-targeted therapies (an area that has been identified as a critical need in PH therapy).

Apelin is a secreted peptide that plays a critical role in cardiac development, angiogenesis, pro-survival signaling, inflammation, and pro-contractile signaling. Our hypothesis is based on preliminary data demonstrating that decreased RV apelin expression in rats with RV failure and in PH patients is a hallmark of maladaptive (decompensated), but not adaptive (compensated) RV remodeling. Furthermore, we found that apelin enhances pro-survival signaling in RV-specific endothelial cells (ECs) and cardiomyocytes (CMs).

Hypothesis:
Our compelling data led us to hypothesize that apelin-mediated signaling will promote a prolonged adaptive remodeling response in the pressure-overloaded RV. This proposal will decipher apelin’s role in the progression of RV failure and, by using a single cell RNASeq approach, identify novel cell-specific targets of apelin-mediated signaling.

Specific Aim 1:
To investigate whether apelin treatment preserves vascular density, contractile function and abrogates the progression of RV failure. We hypothesize that longterm apelin treatment will delay the development of RV failure by stimulating pro-angiogenic and pro-contractile signaling.

Specific Aim 2:
To identify the apelin-mediated transcriptional profile during RV failure development using single cell RNA-Seq. We hypothesize that apelin mediates cell-specific, sex-specific and time-dependent protective responses that delay progression of RV failure.
**Hyaluronan Drives Pathologic Vascular Metabolism in Pulmonary Hypertension**

**Introduction and Background:**
Pulmonary hypertension (PH) is a complex and incurable cardiovascular disorder that is both deadly and morbid. Contemporary treatment rests upon supportive care and maximal relief of pulmonary vasoconstriction. However, disease-modifying therapies that can halt or reverse vascular remodeling, a cardinal feature of PH, are lacking. Mounting evidence indicates that vascular remodeling in PH involves crosstalk between metabolically defective vascular wall cells and their abnormal extracellular matrices.

This basic and translational study examines the impact of hyaluronan (HA), the extracellular matrix glycan, on aberrant vascular metabolism and growth responses in pulmonary hypertension (PH). This project will confront a critical gap in knowledge about the interface between cellular and matrix metabolism in pulmonary vascular biology. The biochemical foundation for this proposal is found in the structure of HA, which is formed by the linear polymerization of aminosugars generated through the hexosamine biosynthetic pathway (HBP). The HBP directly connects to and orchestrates glucose, glutamine, fatty acid, and nucleotide metabolism. Derangements in these metabolite families have been implicated in the pathogenesis of PH.

**Hypothesis and Objectives:**
The proposed studies explore the hypothesis that excessive HA synthesis and HBP activation exacerbate metabolic reprogramming and proliferation of pulmonary artery smooth muscle cells to drive PH. Three main objectives test this hypothesis. The first objective will define the dynamics and key regulators of HA synthesis and HBP in human samples and rodent models of PH. The second objective will elucidate the impact of HA on cellular bioenergetics, and the third objective will test the therapeutic potential of a pharmacologic HA inhibitor in experimental PH.

**Specific Aim 1:**
Establish the contribution of the HA-HBP axis to PH pathogenesis and progression.

**Specific Aim 2:**
Define the impact of the HA-HBP axis on PASMC bioenergetics and phenotype.

**Specific Aim 3:**
Evaluate the therapeutic potential of HA blockade on experimental PH.
Background:
Pulmonary hypertension (PH) is a progressive and potentially lethal syndrome. Unmitigated elevation of pulmonary resistance and pressure leads to right ventricular failure and patient death. Investigations into the molecular mechanisms have defined pathways such as inflammation, smooth muscle cell proliferation, fibrosis, and endothelial cell (EC) dysfunction. A prominent component of EC dysfunction is the formation of plexiform lesions – foci of oblitative/proliferative vascular remodeling with channels lined by myofibroblasts. The origin of the plexiform cells is hypothesized to be endothelial. Yet, the mechanisms of these alterations are not fully understood. Endothelial-to-mesenchymal transition (EndMT) is the process by which EC lose characteristic functions and phenotypic markers and may become migratory contributing to vasculopathy. Transitioning cells with a disruption in cell surface and adhesion proteins may detach from the established vascular monolayer and migrate into the extracellular matrix, leading to increased vascular stiffness. Dedifferentiated cells are able to produce collagen and matrix metalloproteases that remodel the extracellular matrix. An early step in the migration of transformed EC is the loss of VE-cad, a key and specific component of EC adherens junctions (AJ). VE-cad adheres adjoining cells and is the most important component of the AJ. Critical to the function of AJ is the association of VE-cad with the actin cytoskeleton. Therefore, changes in the structure or function of the cytoskeleton may interfere with AJ and ultimately the ability of the EC to maintain a competent monolayer. Formins are a highly conserved family that play a major role in the polymerization and maintenance of the actin cytoskeleton. Each formin consists of two highly conserved domains – the formin homology 1 and formin homology 2 domains (FH1 and FH2, respectively) – that begin the formation of actin. The mammalian Diaphanous-related formin 1 (mDia1) appears to be a global control of actin nucleation. Formin activity and the actin cytoskeleton have been linked to AJ integrity. Global disruption of actin nucleation via mDia1 may influence the behavior of VE-cad on the cell surface and be implicated in EndMT.

Aims:
Aim 1: Determine the role of formin mDia1 in pulmonary endothelial adherens junction formation via epifluorescence microscopy.
Aim 2: Analyze the effect of formin mDia1 inhibition on endothelial monolayer barrier integrity.
Aim 3: Characterize the role of formin mDia1 in endothelial-to-mesenchymal transition in the microvasculopathy of pulmonary hypertension in patients with PH.

Results:
We used the small molecule inhibitor of formin homology 2 domains reconstituted in dimethyl sulfoxide (SMIFH2) as the global inhibitor of mDia1 activity in human pulmonary microvascular endothelial cells (HPMEC). The SMIFH2 was titrated to determine the effective but not lethal concentration of 5µM.
Cellular Junction Imaging

Epifluorescence imaging of AJ morphology was completed. Monolayers of HPMEC were cultured on fibronectin coated glass coverslips and exposed to 5µM SMIFH2, hypoxia (FiO2 <3% via nitrogen washout) for 48 hours, or hypoxia for 48 hours 5µM SMIFH2. There appears to be a synergistic effect of hypoxia and SMIFH2 in breakdown of the monolayer at the AJ (Fig 1) at a lower SMIFH2 concentration previously published in epithelial cells.

Protein Expression

The effect of hypoxia and hypoxia then return to room air on mDia1 expression was assessed via protein expression on Western Blot. HPMECs were placed in a hypoxia chamber for 24 or 48 hours, and then returned to room air with refreshed media for 24 and 48 hours. There was no change in the expression of mDia1, and hypoxia alone appears to not alter the expression of this protein. Hypoxia appears to increase phoso-VE cad (Y685) at both 24 and 48 hours, with a trend toward reversal after 24 hours of normoxia.

Fifteen different lines of pulmonary microvascular endothelial cells were obtained from the Pulmonary Hypertension Break Through Initiative – 10 unique lines from patients with idiopathic pulmonary arterial hypertension (IPAH) and 5 unique lines from patients without pulmonary vascular disease. Initial immunofluorescence imaging showed no obvious differences between the cells. IPAH cells did not show evidence of protein expression of smooth muscle cell markers but did show a trend in intrinsically decreased expression of mDia1.

Barrier Function

To determine the effects of formin inhibition on EC monolayer barrier function, the resistance to an electrical current across the monolayer was assayed using Electric Cell-substrate Impedance Sensing (ECIS). Global formin inhibition with SMIFH2 showed an impressive decline in the resistance across the monolayer and an increased permeability to the electrical current. The addition of 48 hours of hypoxia alone did not significantly change the resistance whereas 48 hours of hypoxia followed by 5µM SMIFH2 had a synergistic effect with a greater loss in resistance and barrier function (Fig 2).

Conclusion:

The cytoskeleton proteins formins have an integral role in maintaining endothelial barrier function, their crucial fundamental behavior, through the preservation of AJ via VE-cadherin. Lack of maintaining barrier function and failure of AJ function may allow the cells to begin EndMT. The global formin inhibitor SMIFH2 is cytotoxic in HPMECs at the prior published concentration in epithelial cells, showing that vascular endothelial cells may be more sensitive to the function of formins than epithelial cells. Structurally, hypoxia and formin disruption act synergistically to create abnormalities in the appearance of the AJ with areas of near complete AJ degradation and holes in the monolayer. This may be acquired via targeted phosphorylation of VE-cad at sites known to increase permeability (tyrosine 685). Functionally, formin inhibition leads to decreased integrity of the monolayer as shown by decreased resistance to an electrical current passed through the monolayer. Hypoxia has a synergistic affect with increased loss of resistance. EC of patients with IPAH may have intrinsically lower levels of mDia1, and this may contribute to endothelial dysfunction in IPAH. Formin activity may be integral to maintenance of the endothelium and resistance to EndMT, and may provide a new target for monitoring and treatment of PH.

**FIGURE 1.**

A and B: Epifluorescence images after 48h of normoxia (A) or hypoxia (B). Hypoxia-exposed cells show increased stress fibers (phalloidin, red) but have preserved intercellular junctions (VE cadherin, green)

C and D: Epifluorescence images after 2 hours of 5µM SMIFH2 (C) showing prominent stress fibers and distraction of VE-cadherin junctions. After 48 hours of hypoxia + 2 hours 5µM SMIFH2 (D), more severe derangements of both VE-cadherin junctions and the actin cytoskeleton are seen, with area of loss of AJ contiguity (box) and separation of the monolayer (arrow). (400x; nuclei are blue).
FIGURE 2. HPMEC monolayer cultured on fibronectin coated 8W10E+ arrays. Monolayers came to steady state of maximal resistance, and then condition applied – control, 5uM SMIFH2 exposure, hypoxia for 48 hours with return to normoxia, or hypoxia for 48 hours then return to normoxia with 5uM SMIFH2 exposure. Data normalized to the preceding one hour of steady-state condition. After an approximate 2 hour period of stabilization, hypoxia: only arrays showed no meaningful change in resistance across the monolayer whereas the SMIFH2 exposure arrays showed a decline in resistance and, more significantly, the pre-exposure to hypoxia and then SMIFH2 exposure has a synergistic effect on reduction in HPMEC monolayer resistance. Each condition includes 8 to 9 repetitions. * delineates p < 0.001.
Background:
We sought to determine the association of body mass index (BMI), intrathoracic VAT, and SAT with PH in patients with advanced lung disease evaluated for lung transplantation using data from the Lung Transplant Body Composition (LTBC) Study. We hypothesized that higher BMI and lower thoracic VAT would be associated with a higher risk of PH.

Aims:
To determine associations of VAT and SAT areas with
1. Pulmonary hemodynamics
2. Vaspin, an adipokine.

Methods:
WA cross-sectional analysis of LTBC study. Adult patients with interstitial lung disease, chronic obstructive pulmonary disease, sarcoidosis, or PH and a chest CT being evaluated for lung transplantation were included. We excluded patients with cystic fibrosis (CF), non-CF bronchiectasis, or those who had a prior lung transplantation.

Logistic regression models were used to assess the association between VAT and SAT and PH adjusted for a priori confounders including age, sex, race/ethnicity, primary lung diagnosis, pulmonary artery wedge pressure (PAWP), and forced vital capacity (FVC). We transformed odds ratio to relative risks and performed mediation analyses to explore whether vaspin mediated the association of adipose tissue with PH using nonparametric bootstrapping estimation methods.

Results:
399 subjects with complete hemodynamics and chest CTs were included; 34% had PH. Doubling of VAT was associated with lower pulmonary vascular resistance (β -0.24, p=0.04) and higher PAWP (β 0.79, p=0.001) after multivariate adjustment. Higher thoracic VAT had decreased risk of PH (RR per doubling of VAT 0.86, 95%CI 0.74-0.99, p=0.04) after multivariate adjustment (Figure 1).

Vaspin (n=78 subjects) was positively correlated with VAT (r 0.38, p=0.001) but not SAT (r 0.02, p=0.86) nor BMI (r 0.08, p=0.47). Vaspin levels were higher in patients without PH as compared to those with PH (median 101.8 [66.5-266.5] vs 92.0 [57.2-175.8] pg/mL, p<0.001, Figure 2); however, vaspin did not appear to mediate the association between VAT and PH.
References:


Mentoring Activities (Mentor to provide a brief update on his/her mentoring efforts and the grant results):
As Dr. Al-Naamani’s mentor, I met with her on a weekly basis throughout the conduct of the study, helping her navigate any issues that arose. Her data and results are fascinating and we are about to submit the manuscript to a high-impact journal. She has already presented an abstract from her data at the American Thoracic Society meeting in 2019 and is actively recruiting for her K23, in large part based on the results from this project.
Selected Final Reports from Recent Awardees – 2016 Award Winner

Olivier Boucherat, PhD
Québec Heart and Lung Institute Research Centre
Québec, QC, Canada
Mentor: Sébastien Bonnet, PhD

Impact of Mitochondrial Heat Shock Protein 90 Inhibition in Pulmonary Arterial Hypertension

Rationale:
Pulmonary arterial hypertension (PAH) is a fatal disease characterized by the elevation of mean pulmonary arterial pressure. PAH is associated with substantial morbidity and premature mortality, exerting a tremendous health and economic impact on patients. Like cancer, pulmonary arterial smooth muscle cells (PASMCs) from PAH patients exhibit a pro-proliferative and anti-apoptotic phenotype leading to development of pulmonary vascular lesions. Targeting this oncogenic-phenotype represents an attractive strategy for treating PAH. In this regard, apoptosis-based therapies used to treat cancer have been tested for the treatment of PAH. Although highly effective anti-tumor drugs have been shown to achieve reversal of the thickening of pulmonary arterioles in models by killing unwanted cells, their usefulness is limited by their relative non-specificity and toxicity to normal cells. To circumvent this problem, the cytotoxic drug must be truly selective. This necessitates identifying and validating a biochemical feature that the target cells possess and which the normal cells lack. Interestingly, the molecular chaperone Hsp90 exists in a subcellular pool compartmentalized in the mitochondria of tumor cells, but not in normal tissues in vivo. In mitochondria, Hsp90 controls tumor cell metabolism and antagonizes cell death, escaping inhibition by conventional small molecule Hsp90 antagonists.

Hypothesis:
Based on similarities between PAH and cancer, we hypothesized that mitochondrial HSP90 (mHSP90) in PAH-PASMCs represents a protective mechanism against stress, promoting their proliferation and resistance to apoptosis.

Results:
We demonstrated that, in response to stress, HSP90 preferentially accumulates in PAH-PASMC mitochondria (dual immunostaining, immunoblot, and immunogold electron microscopy) to ensure cell survival by preserving mitochondrial DNA integrity and bioenergetic functions. Whereas cytosolic HSP90 inhibition displays a lack of absolute specificity for PAH-PASMCs, Gamitrinib (an Hsp90 inhibitor selectively delivered to mitochondria unafflicting Hsp90 homeostasis outside the organelle) decreased mitochondrial DNA content and repair capacity and bioenergetic functions, thus repressing PAH-PASMC proliferation (Ki67 labeling) and resistance to apoptosis (Annexin V assay) without affecting control cells. In vivo, Gamitrinib improves established PAH in two experimental rat models, namely the monocrotaline (MCT) and the Fawn-Hooded rat (FHR) models. Indeed, inhibition of mHSP90 resulted in a significant reduction in mPAP and RVSP compared with vehicle-treated rats. Gamitrinib-treated rats exhibited an increased CO, leading to decreased total pulmonary resistance. Accordingly, pulmonary vascular remodeling of distal PAs was significantly reduced in Gamitrinib-treated MCT and FHRs.

Conclusions:
Our data show for the first time that accumulation of mHSP90 is a feature of PAH-PASMCs and a key regulator of mitochondrial homeostasis contributing to vascular remodeling in PAH. Pharmacological inhibition of mHSP90 using Gamitrinib may represent a promising avenue to improve the clinical outcomes of PAH patients.

The present project has resulted in the following publication:
Biographies

Ronald J. Oudiz, MD
ENTELLIGENCE 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 a past holder of scientific research awards from the American Heart Association and the National Institutes of Health. Dr. Oudiz received the Pulmonary Hypertension Association (PHA) Award of Excellence in Pulmonary Arterial Hypertension (PAH) Care in 2011, the PHA Legacy Award in 2015, and in 2015 he was named a PHA Periwinkle Pioneer for his contributions to the pulmonary hypertension (PH) field. He has authored several papers in the field of PH and has presented his research at national and international seminars. Dr. Oudiz has been on task forces for the past four World Symposia on Pulmonary Hypertension, covering clinical endpoints, diagnostic testing, and right ventricular function and physiology. He is currently the Chair of the American College of Chest Physicians Pulmonary Vascular NetWork, and is Chair-Elect of the PHA's Scientific Leadership Council (SLC). Dr. Oudiz is also a past Editor-in-Chief of the journal Advances in Pulmonary Hypertension. He has participated in several trials of innovative medical treatments for PH, many of which are still ongoing. His research 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
Tufts Medical Center
Tufts School of Medicine
Boston, MA

Dr. Farber has focused on research into pulmonary arterial hypertension (PAH) and the clinical care of PAH patients for over 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.
The ENTELLIGENCE Steering Committee

Biographies

**Mardi Gomberg-Maitland, MD, MSc**  
Professor of Medicine-Cardiology  
Medical Faculty Associates  
Director, Pulmonary Hypertension Program  
George Washington University Medicine and Health Sciences  
Washington, DC

Dr. Gomberg-Maitland earned her medical degree, with special distinction for research in cardiovascular medicine, from the Albert Einstein College of Medicine. She served her residency in internal medicine at New York Presbyterian Hospital/Weill-Cornell Medical Center, and a fellowship in cardiovascular diseases at the Mount Sinai Medical Center. During this fellowship, she was also a visiting fellow in critical care medicine at New York Presbyterian Hospital/Weill-Cornell Medical Center and earned a Master's degree in clinical epidemiology from the Harvard School of Public Health. Her research focus is in understanding the epidemiology of pulmonary hypertension (PH) and development of novel therapeutics and biomarkers. By designing and implementing the largest single-center database, tracking all the patients in the PH clinic, she identified new predictors of survival using simple tests (such as serum creatinine and treadmill testing) and developed a new survival equation to better predict prognosis. She is on multiple international trial steering committees, the lead on drug development at the Pulmonary Hypertension World Congress (JACC publication 2013), and on novel early development trials in early- and late-phase development in PH. She has over 100 publications in top-tier medical journals, including CHEST, Circulation, Circulation Heart Failure, European Respiratory Journal, Journal of American College of Cardiology (JACC), JACC Heart Failure, JAMA Internal Medicine, and the New England Journal of Medicine. She is a section editor at JACC and an associate editor at both CHEST and the European Respiratory Journal.

**Maureen D. Mayes, MD, MPH**  
Professor of Internal Medicine  
Elizabeth Bidgood Chair in Rheumatology  
Division of Rheumatology and Clinical Immunogenetics  
University of Texas – McGovern Medical School  
Houston, TX

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 – McGovern Medical School faculty in 2002 and subsequently established the Scleroderma Clinical and Research Program. Dr. Mayes is the recipient of many distinctions, awards, and grants for the study and treatment of scleroderma. She is the author of over 200 published manuscripts, 28 reviews, 11 book chapters, and 3 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 the founder and director 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.
The ENTELLIGENCE Steering Committee

Biographies

Evangelos D. Michelakis, MD
Professor of Medicine, Division of Cardiology
Vice Chair (Research) – Department of Medicine
Director, Pulmonary Hypertension Program
University of Alberta
Canada Research Chair in Applied Molecular and Mitochondrial Medicine
Edmonton, AB, Canada

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.

Harold I. Palevsky, MD
Professor of Medicine
Perelman School of Medicine of the University of Pennsylvania
Chief, Pulmonary, Allergy and Critical Care Division
Director, Pulmonary Vascular Disease Program
Penn Presbyterian Medical Center
Philadelphia, PA

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 multidisciplinary 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.”
Richard M. Silver, MD
Distinguished University Professor
Medical University of South Carolina
Charleston, SC

Dr. Silver is a graduate of the University of Tennessee and Vanderbilt University School of Medicine. Dr. Silver completed training in internal medicine at the University of North Carolina at Chapel Hill. He trained in pediatric rheumatology with Dr. Barbara Ansell at London’s Northwick Park Hospital, followed by training in adult rheumatology with Dr. Nathan Zvaifler at the University of California at San Diego. Dr. Silver joined the MUSC faculty in 1981 and served as Director of the Division of Rheumatology & Immunology from 1995 to 2018. In 2007, MUSC’s Board of Trustees named him a “Master Teacher” and bestowed the University’s highest academic recognition, “Distinguished University Professor.” He was named the 2007 “Doctor of the Year” by the Scleroderma Foundation. Dr. Silver’s research interests include the pathogenesis and treatment of scleroderma interstitial lung disease, as well as environmental exposures and the risk of systemic sclerosis. He maintains an active practice specializing in all aspects of scleroderma.

Kurt R. Stenmark, MD
Professor of Pediatrics, Medicine, and Anesthesiology
La Cache Endowed Chair and Section Head, Pediatric Critical Care Medicine
Director, Cardiovascular Pulmonary Research
University of Colorado, Anschutz Medical Campus
Aurora, CO

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 is currently Deputy Editor of Pulmonary Circulation and Associate Editor of Cardiovascular Research and on the editorial boards of several journals, including American Review of Respiratory and Critical Care Medicine and Circulation Research. He is a member of the ATS Scientific Advisory Council, Scleroderma Foundation Peer Review, and Pulmonary Vascular Research Institute (PVRI) Steering and Scientific committees. A featured speaker at numerous national and international conferences, Dr. Stenmark has published over 335 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. He has received continuous funding for his research from the NIH since 1984.
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 in the development of pulmonary arterial hypertension 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 (NIH) and Chair of the Pulmonary Circulation Assembly of the American Thoracic Society. He is currently Editor-in-Chief of the journal Pulmonary Circulation, Associate Editor of the American Journal of Physiology-Cell Physiology, and a regular member of the NIH Vascular Cell and Molecular Biology study section.

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