ENTELLIGENCE™
Young Investigators
Award Program
2013

ACTELION
The ENTELLIGENCE program is supported through an educational grant from Actelion Pharmaceuticals US, Inc.
## Table of Contents

- Foreword ................................................................................................. 3
- Program Overview .................................................................................. 4
- Overview of awarded ENTELLIGENCE grants projects ....................... 5
- Abstracts of awarded projects 2012 and 2013 .................................. 18
- Final Reports .......................................................................................... 26
- The ENTELLIGENCE Steering Committee biographies ....................... 40
Dear Colleagues,

We are delighted to announce that in 2013, the Actelion ENTELLIGENCE™ Young Investigators Award Program chose four 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. Since 2005, 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 40 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 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 16 peer-reviewed journals, including Chest, American Journal of Respiratory and Critical Care Medicine, Circulation, PLoS One, Pulmonary Circulation, and American Journal of Physiology. 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.

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, the Young Investigators Award Program will soon begin another cycle of competition, with relevant dates shown below and on the ENTELLIGENCE website: http://entelligencemd.org/.

On behalf of the ENTELLIGENCE Steering Committee, I would like to express our gratitude to Actelion for their generous gifts to the pulmonary vascular disease research community and their ongoing commitment to basic science and clinical research in this arena.

Best regards,

Ronald J. Oudiz, MD

ENTELLIGENCE Young Investigator Award Program Timeline

<table>
<thead>
<tr>
<th>2013</th>
<th>2014</th>
</tr>
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<tbody>
<tr>
<td>Sep</td>
<td>Jan</td>
</tr>
<tr>
<td>Oct</td>
<td>Feb</td>
</tr>
<tr>
<td>Nov</td>
<td>Mar</td>
</tr>
<tr>
<td>LOI Submission</td>
<td>Grant Submission</td>
</tr>
<tr>
<td>Sept. 19 – Nov. 7</td>
<td>Dec. 9 – Feb. 3</td>
</tr>
<tr>
<td>LOI Review</td>
<td>Grant Review</td>
</tr>
<tr>
<td>Nov. 11 – Dec. 9</td>
<td>Feb. 5 – Mar. 4</td>
</tr>
<tr>
<td>Selection Meeting</td>
<td>Notify Applicants</td>
</tr>
<tr>
<td>March 14</td>
<td>March 28</td>
</tr>
</tbody>
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Program Overview

The ENTELLIGENCE™ Young Investigators Award Program
Supporting young investigators

The ENTELLIGENCE Young Investigators Award 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: 8
Awards distributed: 42
Overview of ENTELLIGENCE Awards

Awarded 2013

Harry Karmouty-Quintana, PhD
The University of Texas Health Science Center at Houston
Houston, TX
Mentor: Michael R. Blackburn, PhD
Project Title: The Role of Hyaluronan in Pulmonary Hypertension Associated with Idiopathic Pulmonary Fibrosis (IPF)

Michael L. O’Byrne, MD
Children’s Hospital of Philadelphia
Philadelphia, PA
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

Tien Peng, MD
Hospital of the University of Pennsylvania
Philadelphia, PA
Mentor: Edward Morrisey, PhD
Project Title: The Role of Sonic Hedgehog (Shh) Signaling in Pulmonary Arterial Hypertension

Keivan Zandinejad, MD
Case Western Reserve University
Cleveland, OH
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
Pittsburgh, Pennsylvania
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
Nashville, Tennessee
Mentor: James D. West, PhD
*Project Title: The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension*
Accepted for presentation at 2013 American Thoracic Society Conference

**Kenny Schlosser, PhD**
Ottawa Hospital Research Institute
Ottawa, Canada
Mentor: Duncan J. Stewart, MD
*Project Title: Role of Extracellular Circulating MicroRNAs in Idiopathic Pulmonary Arterial Hypertension*
Accepted for presentation at 2013 American Thoracic Society Conference and presented at 2012 American Heart Association meeting

**Kelly J. Shields, PhD**
Allegheny Singer Research Institute
Pittsburgh, Pennsylvania
Co-Investigator: Joseph M. Ahearn, MD
Mentor: Raymond L. Benza, MD
*Project Title: The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension*
Overview of ENTelligEnCE Awards

2012 Award Winners

From left: Kelly J. Shields, PhD; Eileen Bauer, PhD; Kenny Schlosser, PhD; Joshua P. Fessel, MD, PhD
Overview of Entelligence Awards

Awarded 2011

Jana Bagarova, PhD
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts
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
University of Toronto
Toronto, Ontario
Co-Investigator: Dr. Marc de Perrot
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

Salah Najm, MD
University Hospitals, Case Medical Center
Cleveland, Ohio
Mentor: Kingman Strohl, MD
Project Title: Vascular Reactivity in Response to Acute Hypoxia: Defining Features and Mechanisms

Yon K. Sung, MD
Stanford University School of Medicine
Palo Alto, California
Mentor: Mark Nicolls, MD
Project Title: The Role of Antibodies in the Pathogenesis of Pulmonary Arterial Hypertension
Overview of ENTELLIGENCE Awards

Awarded 2010

Eric Douglas Austin, MD, MSCI
Vanderbilt University School of Medicine
Mentor: James E. Loyd, MD
Project Title: Sex Hormone Abnormalities in Pulmonary Arterial Hypertension
Accepted for publication in Pulmonary Circulation, 2013 and published in Biology of Sex Differences, 2012 and Pulmonary Circulation, 2011
Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2011 and 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

Jason Gien, MD
University of Colorado Health Sciences Center
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
Published in Pediatric Research, 2013
Overview of ENTELLIGENCE Awards

Awarded 2010 (cont.)

Michael J. Passineau, PhD
Allegheny-Singer Research Institute
Mentor: Raymond L. Benza, MD
Project Title: Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin
Published abstract: Molecular Therapy Supplement, 2012
Presented at 2012 American Society of Gene and Cell Therapy Annual Meeting

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
Overview of Entelligence Awards

Awarded 2009

Daniel J. Kass, MD  
University of Pittsburgh  
Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease  
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  
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  
Children’s Hospital Boston  
Brigham and Women’s Hospital  
Boston Adult Congenital Heart and Pulmonary Hypertension Service  
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 Eric Yeager, PhD  
University of Colorado School of Medicine  
Mentor: D. 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 European Respiratory Journal, 2012 and Chest, 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
Published in Arteriosclerosis, Thrombosis, and Vascular Biology, 2011
Presented at 2009 American Heart Association meeting and 2009 American Thoracic Society Conference

Scott D. Halpern, MD, PhD
University of Pennsylvania School of Medicine
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
Published abstracts: American Journal of Respiratory and Critical Care Medicine, 2010 and European Respiratory Journal Supplement, 2010
Published in Respiratory Research, 2011
Overview of ENTELLIGENCE Awards

Awarded 2008 (cont.)

Sanjiv Shah, MD
Northwestern University Medical Center
Mentor: John Varga, MD
Project Title: Generic Risk Factors for Connective Tissue Disease (CTD)-Associated Pulmonary Arterial Hypertension (PAH)
Accepted for publication in Journal of Investigative Dermatology, 2013 and published in 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 Lev 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
John A. Burns School of Medicine
Mentor: Harrison Farber, MD
Project Title: Pulmonary Hypertension due to the 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
UAB School of Medicine
Mentor: David Curiel, MD
Project Title: Downstream Redox Regulation of Endothelin B Receptor in the Pulmonary Endothelium
Published in The Open Gene Therapy Journal, 2008
Overview of ENTELLIGENCE Awards

Awarded 2007 (cont.)

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
Published in Free Radical Biology and Medicine, 2013

Megha H. Talati, PhD
Vanderbilt University Medical Center
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
Published in the American Journal of Physiology: Lung Cellular and Molecular Physiology, 2010
Presented at 2009 American Thoracic Society meeting (travel funded by Cardiovascular Medicine Research and Education Fund)

Yerem Yeghiazarians, MD
University of California, San Francisco
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, PhD, MD
University of Chicago
Mentor: Kenneth Weir, MD
Project Title: Endothelin-Induced Increase in Pulmonary Vascular Smooth Muscle Calcium; The Role of Calcium Channels

Peter Oishi, MD
UCSF School of Medicine
Mentor: Jeffrey Fineman, MD
Project Title: Endothelin-1 Reactive Oxygen Species Interactions in Pulmonary Hypertension

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
Overview of ENTelligence Awards

Awarded 2006 (cont.)

Giuseppe Valacchi, PhD
University of Siena
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, FCCP
Stanford University School of Medicine
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
Pulmonary hypertension (PH) is a disorder affecting the vasculature of the lung that is often associated with idiopathic pulmonary fibrosis (IPF). PH is characterized by increased vascular tone and remodeling of the vasculature, including increased vascular smooth muscle mass and neo-muscularization of vessels. If left untreated, patients die as a result of right ventricular hypertrophy leading to right-sided heart failure. Increased levels of hyaluronan, a component of the lung extracellular matrix, have been observed in patients with pulmonary arterial hypertension. Hyaluronan is produced by hyaluronan synthases (HAS) and can be broken down by hyalurondases into fragments that promote inflammation, remodeling, and angiogenesis through its interaction with hyaluronan binding proteins. However, the involvement of hyaluronan signaling in PH in IPF is not fully understood. Our preliminary data demonstrate a strong correlation between HAS2 expression and mean pulmonary arterial pressure (mPAP) in patients with IPF with and without PH. In addition, increased presence of hyaluronan is observed in remodeled vessels of patients with IPF and PH. Based on these observations our hypothesis is that: Increased hyaluronan deposition in the lungs promotes vascular remodeling in PH associated with IPF. In order to test this hypothesis, we will perform critical proof-of-concept experiments on a unique set of lung tissue derived from lung explants from patients diagnosed with IPF where right-heart catheterization was performed and a diagnosis of PH is available. Human pulmonary artery smooth muscle cells will be used to determine the effect of hyaluronan fragments on cell proliferation and migration. Finally, an experimental model of lung fibrosis and pulmonary hypertension will be utilized to generate pre-clinical data supporting the role of HAS inhibition as a potential therapy to prevent the development of PH in patients with IPF. This proposal will provide key mechanistic and pre-clinical data aimed at enhancing our understanding of how the extracellular matrix is able to participate in vessel remodeling, with the view of developing novel therapies against PH secondary to lung fibrosis.
Pulmonary hypertension is a rare, but extremely morbid condition in children. Hemodynamic measurement obtained via right heart catheterization is an important tool in the diagnosis, classification, and longitudinal care of these patients. However, it appears to be a significant source of iatrogenic mortality. The risk of death in children with pulmonary hypertension appears higher than in children with other forms of heart disease and adults with pulmonary hypertension. The predictors of periprocedural morbidity and mortality are not well defined. We propose to determine the risk factors for right heart catheterization-associated adverse outcomes in a multi-center cohort study and develop a prediction rule based on these factors. We hypothesize that higher catheterization laboratory volume will be associated with lower risk of mortality, and that individual/case level factors (older age, smaller size, general anesthesia, patient status, and etiology of pulmonary hypertension) will increase the risk of adverse outcomes. Our study will utilize administrative data from 40 centers in the United States that contribute data to the Pediatric Health Information System (PHIS) database. All children ages 0-18 years with the diagnosis of pulmonary hypertension who underwent heart catheterization between 2007 and 2012 at a PHIS center will be included. We will exclude patients undergoing electrophysiology studies. Our primary outcome will be mortality within 24 hours of the catheterization and initiation of mechanical circulatory support. Identification of modifiable risk factors provides an opportunity to intervene and improve safety of catheterization in children with pulmonary hypertension and to identify centers of excellence in the field.
Tien Peng, MD
Hospital of the University of Pennsylvania
Philadelphia, PA

The Role of Sonic Hedgehog (Shh) Signaling in Pulmonary Arterial Hypertension

The recapitulation of embryonic programs characterizes a variety of diseases that manifest abnormal cellular proliferation. Unraveling the biological complexity of embryonic vascular development has the potential to provide better understanding of the pathogenesis of adult vascular diseases such as pulmonary arterial hypertension (PAH). Sonic Hedgehog (Shh) is a master regulator of tissue-tissue interaction and cell fate during both heart and lung development in utero. In my preliminary studies, I demonstrated that Hedgehog signaling remains active in the adventitial layer of the adult pulmonary vasculature, and Hedgehog-activated adventitial cells proliferate to generate vascular smooth muscle in an animal model of PAH. Based on these data, I propose that Shh promotes pulmonary vascular remodeling in PAH by activating adventitial proliferation, and subsequent adventitial differentiation into vascular smooth muscle. I will address this hypothesis using both genetic and pharmacologic inhibition of Hedgehog to define Shh’s role in an animal model of PAH.
Pulmonary hypertension (PH) frequently complicates and worsens the course of patients with advanced lung diseases. And despite many years of research, the interventions for PH remain more palliative than curative.

Chronic alveolar hypoxia associated with these diseases is a major factor in the characteristic alterations in the pulmonary and systemic circulations. Although the exact molecular mechanisms responsible for initiating and propagating these processes are not well understood, PH is recognized as having impairments in nitric oxide (NO) signaling. However, current NO-based therapies (inhaled NO and sildenafil) act exclusively via cGMP pathways, even as it is now well-accepted that the vast majority of NO’s cellular activities are mediated through protein S-nitrosylation; the covalent modification of cysteine thiols to form S-nitrosothiols (SNOs).

Disruption of S-nitrosylation is an important component in a number of pathologic conditions, particularly in disease states characterized by disruptions in oxygenation. This includes PH, where we have previously documented reduced SNO/NO bioactivity; notably, levels of S-nitrosylated hemoglobin (SNO-Hb; the main regulator of oxygen delivery) were inversely correlated with disease severity. At the same time, because of the wide spectrum of activities regulated by SNOs, resolution of aberrant S-nitrosylation provides an attractive therapeutic target for disease amelioration. Indeed, acute administration of an S-nitrosylating agent to PH patients rapidly restored SNO-Hb levels, reduced pulmonary arterial pressure (PAP), and improved systemic oxygenation.

The current study builds on the earlier work to determine the long-term benefits of S-nitrosylation therapy. We hypothesize that: 1. Disruption of SNO homeostasis in the body caused by exposure to chronic hypoxia results in elevated PAP and other organ dysfunctions seen in hypoxia-induced PH; and 2. Restoration of SNO homeostasis by administration of an S-nitrosylating agent can prevent or reverse these changes. We will test these hypotheses in a rodent model of hypoxia-induced PH. Positive findings may well lead to clinical assessment of the therapeutic efficacy of S-nitrosylating agents to treat human PH patients.
Eileen Bauer, PhD
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

Complement Activation as a Novel Mechanism of Endothelial Activation in PH

Recent data from our laboratory demonstrate a role for the complement system, a major humoral component of innate immunity, in the pathogenesis of pulmonary hypertension (PH). However, the mechanisms by which complement promotes PH are poorly understood. This proposal aims to test the novel hypothesis that genetic deletion of complement components C3 or C5, or inhibition of activated complement, prevents and/or halts the progression of PH by inhibiting endothelial activation. To test this hypothesis I have proposed two specific aims. Aim 1 will explore if activated complement directly causes activation of pulmonary artery endothelial cells in vitro, and will establish a timeline of endothelial activation in vivo in our animal model of hypoxic exposure. Genetic deletion of complement components C3 or C5 will establish a role for complement in mediating endothelial activation in vivo. Aim 2 will focus on determining the therapeutic potential of the complement inhibitor CR2-crry in PH. Preliminary data show promising results when CR2-crry is administered to mice at the beginning of the disease course suggesting that complement inhibition can attenuate chronic hypoxia-induced PH. The first component of this aim will focus on further refinement of the dosage and timing interval to optimize CR2-crry delivery. Based on these results, inhibitor studies will be performed using mice with established PH to test the inhibitor’s therapeutic potential. Completion of the proposed research will give us a stronger foundation upon which to 1) further investigate the role of innate immunity in PH and 2) further explore the therapeutic potential of drugs targeting the complement system.
2012 Abstracts

Joshua P. Fessel, MD, PhD
Vanderbilt University Medical Center
Nashville, Tennessee

The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a progressive, incurable, and fatal disease of the lung vasculature characterized by increasing pulmonary vascular resistance (PVR) that ultimately leads to right ventricular failure and death. Although the gene responsible for the majority of cases of heritable PAH – bone morphogenetic protein receptor type 2 (BMPR2) – was identified over a decade ago, and despite creation of a robust transgenic mouse model, the precise molecular etiologies of PAH remain unclear. Increasingly, disrupted metabolic processes have been implicated as being key pathologic processes leading to PAH. Decreased insulin sensitivity, impaired glucose homeostasis, and increased aerobic glycolysis have all been demonstrated in PAH in cell culture, in animal models, and in patients with disease. We have recently analyzed the entire metabolome of human pulmonary endothelial cells expressing disease-causing BMPR2 mutations and have shown that many interconnected metabolic pathways are disrupted in PAH. These widespread and interconnected changes suggest the possibility of one or more master regulators that coordinate the balance of cellular metabolic flux and that may be dysfunctional in PAH. Sirtuins are class III lysine deacetylases that have been shown to regulate inflammation, transcriptional activation, and cellular metabolism. Many of the specific pathways regulated by sirtuins align very closely with the metabolic changes we and others have observed in PAH. We thus hypothesize that lysine hyperacetylation resulting from decreased sirtuin function drives the metabolic defects underlying PAH. The proposed studies will demonstrate decreased sirtuin function in cell culture, in transgenic mouse models of PAH, and in cells and tissues from PAH patients. These studies will also use manipulation of sirtuin function (e.g., using knockout mice, caloric restriction, and nutrient excess) to show that sirtuins directly impact disease course in PAH. Demonstration of a causative role for decreased sirtuin function would allow for targeting sirtuins and the downstream metabolic defects to have a potentially disease-modifying effect.
Role of Extracellular Circulating MicroRNAs in Idiopathic Pulmonary Arterial Hypertension

Idiopathic pulmonary arterial hypertension (IPAH) is characterized by a deterioration of the underlying structure of the lung vasculature, and the resulting increase in pulmonary vascular resistance leads to right heart failure and premature death. Despite improvements in treatment, the overall prognosis for IPAH remains poor with no known cure. Although the precise cause of IPAH remains unclear, there is increasing interest in small non-coding RNA molecules known as microRNAs (miRNAs). MiRNAs associate with specific protein complexes and control gene expression by directing the translational inhibition or degradation of target messenger RNAs. To date, over 1000 highly conserved mammalian miRNAs have been annotated, and many have been shown to act as key regulators of fundamental biological processes, including cell proliferation, apoptosis, and inflammation; these processes have all been implicated as possible pathobiologic mechanisms of IPAH. MicroRNAs have traditionally been thought to exist and function exclusively within cells; however, stable extracellular miRNAs have recently been discovered in the blood, which has led to speculation of an entirely new type of paracrine and/or hormonal function. These circulating miRNAs have been isolated from blood plasma under both normal and pathophysiological conditions, including various cancers and cardiovascular disease, but their identification and functional significance in lung vascular diseases like IPAH have not been investigated. We hypothesized that IPAH is associated with aberrant levels of circulating miRNAs which reflect disease-specific mechanisms of vascular injury and/or remodeling. We aim to 1) characterize the global plasma miRNome of a cohort of IPAH patients, 2) identify specific plasma miRNAs with aberrant expression patterns that are conserved between human IPAH and the SUS416/hypoxia rat model, and 3) determine if these miRNAs play a causal, adaptive, or bystander role in the development of PAH, by evaluating the effects of both miRNA inhibition and supplementation in the experimental PAH model. The characterization of these circulating miRNAs may provide new biomarkers of PAH, insight into novel mechanisms underlying the pathobiology of this disease, and potential targets for therapeutic intervention.
The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a rare disease characterized by ever increasing pulmonary vascular resistance and significant vascular remodeling. Although the classic indicators of PAH progression are well recognized, the initiating factors involved in the pathogenesis of PAH are not well understood. Perivascular inflammatory cells have been shown to influence plexiform lesion development, leading to growing interest in the relationship between perivascular inflammation and pulmonary artery (PA) remodeling. Smaller visceral adipose depots are receiving increased attention for their localized inflammatory role in cardiovascular disease. We previously found complement proteins C3 and C4 (C3/C4) deposited at the external elastic lamina of the descending aorta extending through the perivascular adipose tissue (PVAT) in the absence of luminal deposition or plaque development. We determined that C3/C4 bind to collagen and elastin within the vascular wall of murine aorta, suggesting that complement may play a critical role in the pathogenesis of vascular stiffness and atherosclerosis through a mechanism initiated at the adventitia or the PVAT rather than the endothelial surface. The same pro-inflammatory environment may exist surrounding the PAs, contributing to PAH.

We hypothesize that the vascular remodeling due to PAH progression is associated with dysfunctional PA PVAT. We propose that a PAH rat model will have a greater volume of PA PVAT with more extensive C3/C4 deposition and pro-inflammatory protein expression than a control model.

First we will quantify the volume of PA PVAT using microCT and correlate these findings with the extent of vascular remodeling and PA PVAT hypertrophy due to PAH progression as measured through morphological changes using scanning electron microscopy. Second, we will characterize the deposition of C3/C4 in the PA vascular wall and PA PVAT using established immunohistochemistry and histology techniques while evaluating the association with vascular remodeling. Finally, we will identify unique and novel pro-PAH proteins and inflammatory cell populations found in the PA PVAT using proteomics and molecular histology and we will correlate these findings with vascular remodeling at specified time intervals.
Sex Hormone Abnormalities in Pulmonary Arterial Hypertension

Background

Pulmonary arterial hypertension (PAH) is a progressive, fatal disease characterized by increased pulmonary vascular resistance and arterial pressure that results in right heart failure and rapid death. Most types of PAH, including heritable (HPAH) and idiopathic (IPAH), predominantly affect women for unknown reasons, and growing data suggest that sex hormone alterations mediate this discrepancy. In addition, our preliminary laboratory data support the central hypothesis that sex hormone variation modifies disease expression in PAH, with metabolites that possess greater estrogenic effects (16α-hydroxyestrone [16α-OHE$_1$]) in abundance compared to those with less estrogenic effects (2-hydroxyestrogens [2-OHE$_{1/2}$]). To test that hypothesis, we initiated a comprehensive study to determine whether mediators of sex hormone activity are associated with HPAH and IPAH in human females and males. We also sought to understand the underlying biology that drives the relevance of sex hormone metabolism, and questioned whether there might be direct regulation of bone morphogenic protein receptor type 2 (BMPR2) expression by estrogens. We also tested the hypothesis that estrogens directly regulate BMPR2 expression.

Specific Aims

Specific Aim 1. To determine whether sex hormone metabolites with higher estrogen activity are associated with PAH. We hypothesize that a low 2-OHE$_{1/2}$:16α-OHE$_1$ ratio will be associated with an increased risk of PAH (IPAH and HPAH) and more severe disease compared to healthy Controls and BMPR2 mutation carriers (BMCs). In both female and male BMCs, we will perform a matched case-control study comparing patients with PAH to healthy controls with and without BMPR2 mutations. We will determine urinary levels of estrogen metabolites, metabolic ratios, and determine the relative odds of disease.
Specific Aim 2. To determine whether estrogens directly regulate BMPR2 gene expression. Given the importance of BMPR2 activity to HPAH, and possibly IPAH and other forms of PAH, we will test the hypothesis that estrogens and estrogen metabolites alter BMPR2 expression directly, and how this regulation may occur.

**Results**

One hundred and ninety human subjects have been enrolled to date with the urinary estrogen assays completed for 140 subjects, with 17 excluded because phenotypic analysis could not definitively confirm Group 1 PAH.

Female PAH patients and female Controls were not statistically different in terms of age, weight and height, age at menarche, number of pregnancies, parity, or menstrual status. Male PAH patients and male Controls were not statistically different in terms of age, weight, or height. Urine creatinine levels were not statistically different between Cases and Controls for both gender groups.

Estrogen metabolite comparisons were stratified according to gender. For our primary endpoint of interest, the 2-OHE\(_{1/2}\):16\(\alpha\)-OHE\(_1\) ratio, there was a statistically significant difference among females (P = 0.046) with values among Cases (n = 73; 1.11 ng/mg creatinine/ml ± 0.85 SD) lower than Controls (n = 12; 1.60 ng/mg creatinine/ml ± 0.98 SD) (Figure 1). While different, independent comparisons were not statistically significant for the 2-OHE\(_{1/2}\) and 16\(\alpha\)-OHE\(_1\) metabolites among females. There was no statistically significant difference in the sum of urinary metabolites between female Cases and Controls.

For our primary endpoint of interest among males, the 2-OHE\(_{1/2}\):16\(\alpha\)-OHE\(_1\) ratio, there was not a statistically significant difference overall (P = 0.338), although values among Cases (n = 27; 0.91 ng/mg creatinine/ml ± 0.51 SD) were lower than Controls (n = 10; 1.11 ng/mg creatinine/ml ± 0.57 SD). Among males, the 2-OHE\(_{1/2}\) level was statistically significantly lower in Cases than Controls (P = 0.002; Cases 6.62 ng/mg creatinine/ml ± 4.09 SD vs Controls 15.48 ng/mg creatinine/ml ± 13.15 SD). The 16\(\alpha\)-OHE\(_1\) metabolite level was also lower in Cases than Controls but did not reach statistical significance. There was a statistically significant difference in the sum of urinary metabolites (2-OHE\(_{1/2}\) + 16\(\alpha\)-OHE\(_1\)) between male Cases and Controls (P = 0.018; Cases 16.09 ng/mg creatinine/ml ± 8.46 SD vs Controls 31.62 ng/mg creatinine/ml ± 25.63 SD).

Expression of BMPR2 mRNA by both human lymphocytes and human pulmonary microvascular endothelial cells (PMVECs) was examined after 24 hours of treatment with 1 µM estradiol (E2). Compared to Controls, BMPR2 mRNA expression measured by quantitative PCR significantly decreased after 24 hours of E2 in both normal human lymphocytes (P < 0.05) and PMVECs (P < 0.01) relative to the housekeeping gene HPRT. To explore whether this response was unique to E2, expression of BMPR2 mRNA by PMVECs was examined after 24 hours of treatment with the
16-estrogen, E3 (estriol). Treatment with 10 nM (P = 0.001), 30 nM (P = 0.001), and 100 nM (P = 0.009) of E3 resulted in significantly lower BMPR2 gene expression compared to the unexposed control PMVECs (Figure 2).

We next determined that there is an evolutionarily conserved estrogen receptor binding site in the BMPR2 promoter, and used a gel-shift assay to determine that this binds to the estrogen receptor. Finally, we used transfection of increasing quantities of estrogen receptor alpha (ERα) to demonstrate that ERα contributes to the reduction in expression of BMPR2 by estrogens.

**Conclusion**

Our data support the hypothesis that alterations in sex hormone metabolites associate with PAH, suggesting that metabolites with higher estrogen activity (eg, as represented by a lower ratio of 2-hydroxyestrogens:16α-hydroxyestrogens) are more abundantly expressed among PAH patients compared to controls. Interestingly, there are growing data from murine models demonstrating the detrimental impact of 16-hydroxyestrogens on the development of PAH.

Also consistent with our hypothesis, both the parent compound estrogen (estradiol) and the 16-hydroxyestrogen (estriol) reduce BMPR2 gene and protein expression in vitro; this reduction is mediated, at least in part, via ERα. Given the association of BMPR2 gene mutations with PAH, and that BMPR2 expression is reduced in the lungs of multiple types of PAH patients, reduction of BMPR2 expression via estrogens may provide a mechanistic link to partially explain the gender discrepancy in PAH.

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**Figure 1.** Female PAH patients have a lower 2-OHE$_{1/2}$:16α-OHE$_1$ ratio than healthy Controls (P = 0.046).
(A) BMPR2 expression by quantitative RT-PCR, normalized to HPRT, is reduced in cultured human lymphocytes treated with 1µM E2 compared to untreated controls (P < 0.05). (B) BMPR2 expression by quantitative RT-PCR, normalized to HPRT, is reduced in cultured PMVECs treated with 1µM E2 compared to untreated controls (P < 0.01). (C) BMPR2 expression by quantitative RT-PCR, normalized to HPRT, is reduced in cultured PMVECs treated with varying doses of E3 compared to untreated controls (P < 0.01 for each comparison). (D) Estrogen and induction of proliferation through PMA both independently inhibit BMPR2 expression, by densitometry of northern blot (3 replicates per condition) at P < 0.05 by two-way ANOVA. The combination of proliferation and estrogen produces a ~4x inhibition of BMPR2 expression (P < 0.05 by Student’s t-test with adjustment for multiple comparisons).

Figure 2. Treatment with different estrogens reduces BMPR2 mRNA expression in multiple normal cell types.
Smooth Muscle Cell Related Vascular Remodeling in Pulmonary Hypertension in Congenital Diaphragmatic Hernia

Background

Congenital diaphragmatic hernia (CDH) is a common structural birth defect of failed diaphragm development. The incidence of CDH ranges from 1/2500–1/4000 births and is responsible for 1% of total infant mortality in the United States. Morbidity and mortality of CDH is related to respiratory failure from pulmonary hypoplasia and pulmonary arterial hypertension (PAH) due to abnormal pulmonary vascular development. Defective pulmonary vascular remodeling exacerbates already present PAH in patients with CDH. There is no definitive treatment for PAH in CDH.

Despite considerable clinical and basic science interest, the mechanism of PAH in CDH has yet to be elucidated. One major problem limiting our understanding of this congenital condition has been the lack of a reproducible model of CDH. The Slit3 knockout mouse is a genetically altered mouse model with a congenital diaphragmatic hernia. Slit3 is a matrix protein that is part of the Slit/ROBO family. Slit3 plays a role in axon guidance and repulsion. Slit3 has been shown to promote endothelial cell migration and angiogenesis. While expressed in the pulmonary mesenchyme and pulmonary vascular cells, little is known about the role of Slit3 in smooth muscle cell function, pulmonary circulation development, and pulmonary vascular function. The pulmonary vasculature has not been evaluated in this mouse model for evidence of pulmonary hypertension.

It is plausible that the disruption of Slit3 signaling leads to abnormal smooth muscle cell phenotype and function. Furthermore, abnormal smooth muscle cell function may cause pulmonary vascular remodeling and subsequent development of pulmonary hypertension. The Slit3 -/- mouse provides a model to investigate the role of Slit3 in pulmonary artery smooth muscle cell (PASMC) differentiation and the development of pulmonary hypertension in the context of CDH.
Aims

Hypothesis:
Alterations in Slit3 signaling initiate changes in pulmonary vascular smooth muscle cell phenotype. Aberrant smooth muscle cell phenotype drives pulmonary vascular remodeling responsible for the development of PAH in a mouse model for CDH.

Aim#1: To determine the molecular effects of Slit3 signaling on phenotype diversity, differentiation, and gene expression in PASMCs in vitro.

Aim#2: In vitro characterization of the role of Slit3 upon PASMC migration related to pulmonary vascular remodeling.

Aim#3: To determine the role of Slit3 in the physiologic function and structural development of the pulmonary circulation in a mouse model of CDH (in vivo).

Results

Slit3 influenced the differentiation of smooth muscle cells in culture. To determine if the loss of Slit3 would influence cell differentiation, recombinant Slit3 protein was introduced to Slit3 null mouse PASMC cultures. Smooth muscle cytoskeletal markers and the pluripotent cell marker SCA-1 were determined by RT-PCR. After 7 days, SCA-1 was elevated and the muscle cytoskeletal markers were decreased in the Slit3 null cultures. With the addition of Slit3, SCA-1 levels declined and the cytoskeletal markers increased toward baseline. These results indicated that the loss of Slit3 in PASMCs leads to a Slit3-dependent change in phenotype.

The loss of Slit3 lead to the downregulation of FGF transcripts. To investigate transcriptome-wide changes to PASMCs related to the loss of Slit3, microarray analysis was performed. Analysis revealed enrichment of the following annotation clusters: extracellular region, cell adhesion, cytokine and chemotaxis, blood vessel development, development/morphogenesis, and respiratory system development. The fibroblast growth factor (FGF) proteins FGF2, FGF5, FGF9, and FGF10 all showed significant downregulation. The annotation clusters revealed by the microarray analysis provide insight into potential gene pathways that may be altered due to the lack of Slit3 in smooth muscle cells. The pathways may be important in influencing smooth muscle cell phenotype and function.

The Slit3 KO mice with CDH had increased right ventricular (RV) pressures. RV pressures were measured in Slit3 knock-out (KO, +/-) mice with CDH and compared to wild-type (WT) controls. Measurements were performed by direct puncture of the right ventricle with a fluid-filled catheter.
Mean pressure, pulse pressure, and systolic pressure were all elevated in the Slit3 CDH mice in comparison to WT controls (Figure 1). Our pressure data indicated a significant elevation in ventricular pressures in the Slit3 KO mice with CDH. This is consistent with pulmonary hypertension.

**Slit3 null mouse had decreased distensibility in the pulmonary artery, which is consistent with pulmonary hypertension (Figure 2).** Right pulmonary artery was mounted on a pressure myograph from Slit3 -/-, WT, and heterozygote mice. The vessels were exposed to graded increases in the intraluminal pressure. The vessel diameter was measured in real time. The pulmonary artery from the Slit3 -/- mice had decreased distensibility at all pressures in comparison to WT and heterozygote samples. The differences were statistically significant at all measured pressures. Decreased distensibility is an indicator of altered vessel wall mechanics that occur in a hypertensive state.

**Conclusion**

The goal of my proposal was to determine the signaling mechanisms related to pulmonary vascular remodeling seen in pulmonary hypertension in the context of CDH. Based upon cell culture studies, we have demonstrated, through alterations in the cytoskeletal marker profile, that Slit3 may play a role in smooth muscle cell differentiation. Our in vivo characterization of the Slit3 null mouse with CDH demonstrated increased RV pressures, RV remodeling, alterations in the matrix composition of pulmonary arteries, and decreased vessel distensibility. All of these findings are consistent with pulmonary hypertension. The data derived are important because we demonstrated that Slit3 may play a role in smooth muscle cell differentiation and function. More importantly, the animal data are the first demonstrations of pulmonary hypertension in a viable mouse model for CDH.
Figure 1. Slit3 null mice showed evidence of pulmonary hypertension vs WT controls. Mean pressure (Panel A: 8.2 vs 18.7 mm Hg), systolic pressure (Panel B: 17.5 vs 35.2 mm Hg), and pulse pressure (Panel D: 15.8 vs 29.9 mm Hg) were significantly elevated in SLIT3 -/- mice. Diastolic pressure (Panel C: 1.7 vs 5.3 mm Hg) showed a trend towards elevation in SLIT3 -/- mice. Heart rate (Panel E: 458 vs 464 bpm) was equivalent between KO and WT controls.
Figure 2. Slit3 null mouse had decreased distensibility in the pulmonary artery. Right pulmonary artery was mounted on a pressure myograph from Slit3 -/-, WT, and heterozygote mice. The vessels were exposed to graded increases in the intraluminal pressure. The pulmonary artery from the Slit3 -/- had decreased distensibility at all pressures. The differences were statistically significant at all measured pressures. Decreased distensibility is an indicator of altered vessel wall mechanics present in hypertensive states.
Final Report

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Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin

Background

The overall goal of this proposal is to design and test a gene therapy strategy for pulmonary arterial hypertension (PAH). PAH is a rare, deadly and incurable disease with a mean survival of 2.8 years from onset of symptoms if left untreated. Of the three classes of approved therapeutics, endothelin receptor antagonists, phosphodiesterase inhibitors, and prostacyclins, prostacyclin is the most effective therapy. However, complicated delivery systems and potential side effects associated with the present formulation of prostanoids (eg, prostacyclin) have deterred some patients and caregivers from instituting this highly effective class of agent. The challenge to be addressed by this proposal is the need for a therapeutic regimen that allows for endogenous production of prostacyclin therapy within the patient’s own body, throughout the entire lifetime of the patient.

Because prostacyclin can be produced endogenously through expression of the enzyme prostacyclin synthase (PGIS), gene therapy has previously shown proof-of-principle efficacy in animal models by enabling endogenous production of prostacyclin and reversal of experimental PAH. This proposal will build upon this concept, but will execute this strategy using newly developed gene transfer technology that obviates the viral gene delivery vectors that have been used in prior studies. Viral vectors have been extremely useful in earlier studies, but have limited duration of expression, and due to host immune response, cannot affect life-long therapy, nor can they be re-dosed. Our gene delivery system, which used ultrasound-induced microbubble cavitation to allow entry of non-viral DNA vectors into cells, is thought to evade host immune responses, theoretically allowing re-dosing of the PGIS therapeutic transgene as a periodic booster throughout the entire lifespan of the patient.
An additional innovation of this proposal toward achieving the field-wide goal of endogenous prostacyclin production is the choice of the salivary glands as the therapeutic biosynthesis site. The salivary glands can be accessed through a bloodless, outpatient procedure, and contain a robust endocrine secretory pathway capable of secreting transgene products into the intravascular space. The encapsulated, fixed volume of the intraductal labyrinth of the salivary glands also allow precise control of the delivery system, making ultrasound-assisted gene transfer (UAGT) far more practical and consistent than has been observed in other organs (eg, heart or pancreas).

Aims

1) To deliver the Cox-1/PGIS fusion protein to the submandibular gland of the rat using UAGT and measure serum levels of PGI2 on days 2, 7, and 21 post-treatment.

2) To utilize the Cox-1/PGIS fusion protein as a treatment modality on day 11 post-monocrotaline in the left pneumonectomy/monocrotaline rat model of PAH.

Results

Construction and validation of Cox-1/PGIS fusion protein open reading frame (ORF). The transfer of the Cox-1/PGIS fusion protein cDNA from our collaborators could not be affected. We therefore undertook synthesizing the ORF for this fusion protein, and have succeeded in doing so. We obtained a mammalian expression vector wherein the Cox-1/PGIS fusion protein cDNA is driven by a CMV (cytomegalovirus) promoter.

In vivo validation of fusion protein function. The Cox-1/PGIS-expressing plasmid was transfected to HEK293 cells in vitro and media was collected 48 hours later. Cells transfected with a GFP-expressing plasmid were used as a negative control. Our initial attempts to detect 6-K-P in the media were unsuccessful, but we later learned that extraction of the 6-K-P from the sample with a C18 column allowed us to detect 6-K-P with ELISA (enzyme-linked immunosorbent assay; Cayman Chemical, Ann Arbor, MI). Figure 1 shows these in vitro results.

In vivo validation of fusion protein expression and function. In order to generate maximum 6-K-P signal in the serum, we undertook hydrodynamic gene delivery of the Cox-1/PGIS-expressing plasmid to rats and collected blood 48 hours later. Figure 2 shows results of these studies in which we detected Cox-1/PGIS in the liver of treated animals, and observed a resultant increase in serum 6-K-P.
Figure 1. Function of Cox-1/PGIS fusion protein in vitro. The Cox-1/PGIS-expressing mammalian expression vector was transfected to HEK293 cells and media was harvested 48 hours later. Media from GFP-transfected cells was used as a negative control. 6-K-P ELISA was performed following a C18 column extraction step.
**Figure 2.** Expression and function of Cox-1/PGIS in vivo. A) Western blot probed with anti-PGIS antibody. Lysates from Cox-1/PGIS-transfected cells were used as positive control. Lysates from two untreated (-) mice were used as negative controls, and one animal (+) receiving hydrodynamic gene delivery of the Cox-1/PGIS-expressing plasmid showed a PGIS-immunoreactive band of the appropriate size to be the Cox-1/PGIS fusion protein. B) Blood collected from a hydrodynamically-treated animal 48 hours after gene delivery of either Cox-1/PGIS or an untreated control was assayed for 6-K-P with an ELISA following a C18 extraction step.
Expression of Cox-1/PGIS in the salivary gland using UAGT resulted in serum 6-K-P levels that were not statistically different from baseline. 8 animals were treated with Cox-1/PGIS using UAGT to the salivary gland, with 2 animals showing a trend toward elevated 6-K-P that was not statistically significant (data not shown). Expression of the Cox-1/PGIS mRNA was measured with RT-PCR (reverse transcriptase-polymerase chain reaction), and showed levels <1/100 of liver-directed expression.

Conclusion

• The Cox-1/PGIS fusion protein ORF we constructed expressed a functional version of the fusion protein.
• Cox-1/PGIS expression in the liver of rats resulted in therapeutic levels of circulating PGI2, as measured by its stable metabolite, 6-K-P.
• UAGT to the salivary gland resulted in overall low levels of expression of Cox-1/PGIS that did not raise serum levels of 6-K-P above baseline.
• Future applications of this research include the following: 1) While hydrodynamic gene transfer of Cox-1/PGIS to the human liver may not be clinically practicable, hydrodynamic gene transfer to skeletal muscle may be, and may represent a path forward for this therapeutic approach; 2) UAGT to the salivary gland will need to be more consistent and result in higher levels of expression before it can be a practical approach to this treatment strategy for PAH.
Ronald J. Oudiz, MD, FACP, FACC, FCCP 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. Dr. Oudiz 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 immediate 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.
Dr. Harrison (Hap) 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.

Dr. Farber serves on many panels for the development of clinical recommendations in PAH, has participated in large multicenter clinical trials, and is 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 ENTRELLIGENCE Steering Committee

Biography

Adaani E. Frost, MD
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Dr. Adaani Frost is Professor of Medicine in the Pulmonary and Critical Care Section of Baylor College of Medicine, Houston, Texas. She undertook her postgraduate training in pulmonary and critical care, including a fellowship in lung transplantation in the Toronto Hospital System and McGill University. She was Medical Director of the Lung Transplant Program at both the Methodist Hospital and St. Luke’s Episcopal Hospital from 1990 to 2001 and has since developed the Pulmonary Hypertension and Advanced Lung Disease Service at Baylor. Currently, she is involved in clinical management and clinical research on patients with end stage lung disease, predominantly in pulmonary hypertension, and pulmonary fibrosis. Dr. Frost was on the Scientific Advisory Council of the Pulmonary Hypertension Association until 2009, is on the steering committee of REVEAL (a US-based registry of more than 3500 pulmonary hypertensive patients), has authored numerous papers on pulmonary hypertension, and is a participant in multiple new and ongoing studies in the treatment of pulmonary hypertension.
Mardi Gomberg-Maitland, MD, MSc, is Associate Professor of Medicine and Director of the Pulmonary Hypertension Program at the University of Chicago Medical Center in Chicago, Illinois. Dr. Gomberg-Maitland 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, Dr. Gomberg-Maitland 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 100 published manuscripts, 19 reviews, 5 book chapters and 1 full length book. 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 ‘Two-Stage Genome-Wide Association Study in Scleroderma’ that has the dual objectives to identify genes that influence disease susceptibility and severity, as well as to serve 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 Pulmonary Hypertension and the Chair-Elect of the 3-CPR Council of the American Heart Association, and he serves on the editorial boards of both Circulation and Circulation Research. Recently, 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.
Biography

Harold I. Palevsky, MD
Professor of Medicine
University of Pennsylvania
Chief, Pulmonary, Allergy and Critical Care
Director, Pulmonary Vascular Disease Program
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Harold I. Palevsky, MD, is a Professor of Medicine at 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, lung transplant evaluation, 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.”
Ivan M. Robbins, MD, is Associate Professor of Medicine, Department of Medicine, Division of Allergy, Pulmonary, and Critical Care Medicine and Director of the Pulmonary Vascular Center at Vanderbilt University Medical Center.

Dr. Robbins earned a medical degree from Case Western University School of Medicine, completed an internship and residency at Metrohealth Medical Center, and pursued a Pulmonary and Critical Care fellowship from Vanderbilt University School of Medicine.

An internationally recognized expert in the field of pulmonary vascular disease, Dr. Robbins’ research interests include the mechanisms of action of epoprostenol and the role of oxidant stress in pulmonary arterial hypertension. He has been published in numerous peer-reviewed journals, including Circulation, American Journal of Respiratory and Critical Care Medicine, and the Journal of the American College of Cardiology.
Dr. Richard Silver serves as Director of the Division of Rheumatology & Immunology at the Medical University of South Carolina (MUSC). He was born in Tennessee and graduated from the University of Tennessee–Knoxville. After graduating from Vanderbilt University School of Medicine in 1975, Dr. Silver completed training in Internal Medicine at the University of North Carolina-Chapel Hill, and then in Rheumatology at London’s Northwick Park Hospital and at the University of California-San Diego. He joined the faculty at MUSC in 1981, where currently he is Professor of Medicine and Pediatrics and is the Director of the Division of Rheumatology and Immunology. In 2007, MUSC’s Board of Trustees named him a “Master Teacher” and bestowed the University’s highest academic recognition, Distinguished University Professor. Also in 2007, the Scleroderma Foundation named him their “Doctor of the Year.” Dr. Silver’s major research interest is interstitial lung disease associated with systemic sclerosis.