Chairman’s Note

I am pleased to welcome you to the latest issue of Spotlight.

Each year, the ENTELLIGENCE™ Young Investigators Award Program provides funding to promising young investigators to encourage and promote quality medical care and enhance patients’ lives through research in pulmonary vascular diseases (PVDs).

As we prepare for the 8th review cycle for the Young Investigators Award (YIA) Program, we’d like to acknowledge the generous ongoing support of Actelion. Since 2005, when the program began, a total of 38 young investigators from around the United States and Canada have received grant awards for pulmonary vascular disease research.

We hope you enjoy this issue, in which we’ve included interviews with 2012 award winners Joshua Fessel, MD, PhD, and Eileen Bauer, PhD. We also report on the career advances of some of our other talented award winners.

Warm regards,
Ronald J. Oudiz, MD, Program Chairman

Table of Contents

Chairman’s Note ........................................ p. 1
2012 YIA Award Recipients .............. p. 1
Joshua P. Fessel, MD, PhD ........... p. 2
Eileen Bauer, PhD .............. p. 3
ENTE LLIGENCE 2012-2013 YIA Program Timeline .......... p. 4
2013 Conferences ......................... p. 4
Steering Committee ..................... p. 4
ENTE LLIGENCE YIA ‘Fast Facts’........ p. 4
Recent Promotions ...................... p. 4

2012 Young Investigators Award Recipients

Eileen Bauer, PhD
University of Pittsburgh
Co-Investigator: Stephen Tomlinson, PhD
Complement Activation as a Novel Mechanism of Endothelial Activation in PH

Kenny Schlosser, PhD
Ottawa Hospital Research Institute
Role of Extracellular Circulating MicroRNAs in Idiopathic Pulmonary Arterial Hypertension

Joshua P. Fessel, MD, PhD
Vanderbilt University Medical Center
The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension

Kelly J. Shields, PhD
Allegheny Singer Research Institute
Co-Investigator: Joseph M. Ahearn, MD
The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension
Dr. Fessel is a 2012 YIA Program winner for his project, The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension. He is a Clinical Fellow in Pulmonary and Critical Care Medicine at Vanderbilt University Medical Center in Nashville, TN. Spotlight spoke with him recently about receiving the award and his future plans.

Spotlight: What is the biggest reward associated with winning the ENTELLIGENCE™ YIA award? 
**Dr. F:** The biggest reward is concrete external validation of our hypotheses regarding molecular metabolism and the contribution of metabolic reprogramming to the development of pulmonary arterial hypertension (PAH). Additionally, having funds available to test these hypotheses is clearly a major benefit.

Spotlight: What is the major challenge you face in conducting your ENTELLIGENCE YIA project? 
**Dr. F:** The major challenge centers on the development of methods and reagents. Many sirtuin assays commercially available are designed for evaluation of activators or inhibitors in a relatively simple matrix, as opposed to activity measurements from biological samples. This requires modification of the available protocols and validation of these modifications. We are working with a number of transgenic mouse models, simultaneously developing, phenotyping, and conducting experiments with these animals. Finally, we are incorporating more sophisticated methods of metabolic analysis into our experiments, many of which are new to our laboratory. These challenges have actually opened new avenues of inquiry.

Spotlight: What are your short- and long-term professional goals? 
**Dr. F:** I anticipate obtaining a tenure-track faculty position within the next year and securing career development funds to transition to independence. I will continue investigations of metabolic reprogramming at the molecular and cellular level in PAH, looking to understand not only the molecular details of metabolic control in PAH but also the translation of these findings to patients. Pursuing these investigations through to identification of novel therapeutic strategies is a major professional goal. In the long-term, I would like to extend analyses of molecular metabolism into other disease states.

**Abstract**

The Role of Sirtuins and Lysine Acetylation in Pulmonary Arterial Hypertension

**Background:** 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. The precise molecular etiologies of PAH remain unclear. Increasingly, disrupted metabolism and metabolic reprogramming have been implicated as key pathologic processes leading to PAH. We 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 1) the possibility of one or more master regulators coordinating the balance of cellular metabolic flux and 2) these regulators 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 observed in PAH.

**Hypothesis:** We hypothesize that decreased sirtuin function (and the resulting lysine hyperacetylation) drives the metabolic defects underlying PAH.

**Materials and Methods:** We will demonstrate decreased sirtuin function and increased lysine acetylation 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 (eg, using mice with specific sirtuins either knocked out or overexpressed, caloric restriction to activate sirtuins, and nutrient excess to decrease sirtuin activity) to show that sirtuin function directly impacts the disease course in PAH.

**Aims:** The aims of the proposed studies are as follows:

1. To demonstrate decreased sirtuin activity and resultant lysine hyperacetylation in a specific model of PAH (ie, the BMPR2 mutant model).
2. To show that impairing sirtuin function will make BMPR2-mediated PAH worse (specifically with regards to the metabolic and cardiovascular phenotypes), and that increasing sirtuin function will ameliorate disease.
3. To confirm relevance to human disease by showing decreased sirtuin activity in patients with PAH.

**Implications:** By showing that sirtuins play a key role in the pathogenesis of PAH, we will identify these enzymes as a new drug target with the potential to develop true disease-modifying therapies. Currently, no such therapies exist for PAH. In the process, we will also show that metabolic reprogramming is a central pathogenic process in PAH, providing additional avenues for therapeutic development.
Spotlight on 2012 YIA Award Recipients*

Eileen Bauer, PhD

Dr. Bauer is a 2012 YIA Program winner for her project Complement Activation as a Novel Mechanism of Endothelial Activation in PH. She is a Research Instructor at the University of Pittsburgh School of Medicine in Pittsburgh, PA.

Spotlight: What is the biggest reward associated with winning the ENTELLIGENCE™ YIA award?

Dr. B: With government funding so tight, many good and innovative research ideas from scientists in all fields remain unfunded. My proposal, which states that the innate immune system contributes to pulmonary hypertension (PH), is not in the mainstream. Thus, the biggest reward is the opportunity to pursue these novel studies and, hopefully, shed some light on a largely neglected area of PH research.

Spotlight: What is the major challenge you face in conducting your ENTELLIGENCE project?

Dr. B: The project involves the exposure of mice to chronic hypoxia to induce PH. While we use this model routinely in our lab, I expect to encounter some difficulties in trying to get all the antibodies to work successfully on the lung tissue while doing a time-course hypoxia study.

Spotlight: What are your short- and long-term professional goals?

Dr. B: For the short term, I am focused on exploring in greater detail the role of innate immunity in PH. I hope this work will lead to many publications so I can reach my long-term goal of getting solid funding to start my own independent laboratory.

Abstract

Complement Activation as a novel mechanism of endothelial activation in pulmonary hypertension (PH)

Background: Pulmonary arterial hypertension is a progressive disease with high mortality. Mortality results from right heart failure that develops in response to elevated pulmonary arterial pressures due to increased vasoconstriction and remodeling of the pulmonary vasculature. The limited success of current therapies led us to focus on the role of the immune system in PH. The importance of the complement system as part of the innate immune system is well established and studied in many immune related diseases. The complement system functions to fight infection and can interact with the coagulation cascade to amplify endothelium, platelet, and immune cell activation. Its activation not only leads to killing of its target cell but also often involves damage and death to adjacent cells. The beneficial effect of the complement system is therefore only achieved under tight regulation and limited activation. Our lab recently observed increased complement deposition in human and experimental PH. Furthermore, preliminary data demonstrate that inhibition of complement activation using the targeted inhibitor CR2-crry limits the development of PH in an animal model of chronic hypoxic exposure. Targeted inhibition at the site of complement activation by CR2-crry has shown success in mouse models of intestinal ischemic reperfusion, ischemic stroke, or collagen-induced arthritis.

Hypothesis: Targeted therapeutic inhibition of activated complement prevents and/or halts the progression of hypoxia-induced PH in mice.

Material and Methods: In vitro experiments involve the use of Human Pulmonary Endothelial Cells which will be treated with complement C3a, C5, or hypoxia over time, focusing on endothelial activation as the endpoint. Endothelial activation will be assessed by flow cytometry with appropriate antibodies. In vivo experiments in mice involve hypoxic exposure, leading to the development of PH after 21 days, as assessed by measuring right ventricular pressures, right ventricular hypertrophy, and pulmonary vascular remodeling. Time course experiments in hypoxia will be used to determine a time line of complement activation and endothelial activation in this model.

Aims: The first aim will test the hypothesis by assessing whether the pulmonary endothelium is activated by complement in vitro as well as in vivo in hypoxia-induced PH. The second aim will explore whether the complement inhibitor CR2-crry can halt or reverse the development of hypoxia-induced PH.

Implications: Currently, the role of the immune system in the pathogenesis of PH is poorly understood and despite the growing number of therapeutics, PH remains a progressive and fatal disease. 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.

*Other YIA Award recipients will be presented in future issues of Spotlight.
## ENTELLIGENCE™ YIA Steering Committee

- **Ronald J. Oudiz, MD**  
  *Program Chairman*  
  LA Biomedical Research Institute at Harbor-UCLA Medical Center

- **Harrison (Hap) Farber, MD**  
  Boston University School of Medicine

- **Adaani E. Frost, MD**  
  Baylor College of Medicine

- **Mardi Gomberg-Maitland, MD, MSc**  
  University of Chicago Medical Center

- **Maureen D. Mayes, MD, MPH**  
  The University of Texas Health Science Center at Houston

- **Evangelos D. Michelakis, MD**  
  University of Alberta

- **Harold I. Palevsky, MD**  
  University of Pennsylvania

- **Ivan M. Robbins, MD**  
  Vanderbilt University Medical Center

- **Richard M. Silver, MD**  
  Medical University of South Carolina

## YIA Program Fast Facts

- Year established: 2005
- Review cycles completed: 7
  - Awards distributed: 38
  - Funding: $3,175,000
- YIA winners (2005-2012)
  - Presentations at scientific congresses: 25
  - Peer-reviewed manuscripts: 15
  - Published abstracts: 9
  - Book chapters: 1

## YIA Award Winners: Career Advances

- **Gaurav Choudhary MD**  
  Associate Professor of Medicine (2012)

- **Hyung Chun, M.D**  
  Assistant Professor of Medicine* (2009)

- **Anna R. Hemnes, MD**  
  Assistant Professor of Medicine* (2009)

- **Marco Mura, MD, PhD**  
  Assistant Professor of Medicine (2012)

- **Michael J. Passineau, MD**  
  Director, Gene Therapy Program* (2010)

- **Meredith A. Preuss, PhD**  
  Administrative Director (2010)

- **Sanjiv J. Shah, MD**  
  Associate Professor of Medicine* (2012)

- **Ramana Sidhaye, MD**  
  Assistant Professor of Medicine (2009)

- **Michael E. Yeager, PhD**  
  Assistant Professor* (2011)

*PVD-related position

## ENTELLIGENCE Young Investigator Award Program Timeline

<table>
<thead>
<tr>
<th>Event</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant submission</td>
<td>Dec 19 – Feb 1, 2013</td>
<td>Feb 5 – 25, 2013</td>
</tr>
<tr>
<td>Grant review</td>
<td></td>
<td>Mar 15, 2013</td>
</tr>
<tr>
<td>Selection meeting</td>
<td></td>
<td>Mar 15, 2013</td>
</tr>
<tr>
<td>Notify applicants</td>
<td></td>
<td>Mar 25, 2013</td>
</tr>
</tbody>
</table>