



P A R K E R B . F R A N C I S  
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## **2008 PBF Fellows**

# **FINAL PROJECT SUMMARIES & PUBLICATIONS DURING THE PBF FELLOWSHIP**

**May 2011**

The 2008 PBF Fellows submitted their final progress reports for a May 30, 2011 deadline. As part of the final report, the PBF Fellows are asked to provide a summary of their PBF-funded project in language understandable to a non-scientist. The summary should outline the progress made in the aims of their PBF Fellowship application and describe how their research may benefit patients with lung disease.

The 2008 PBF Fellows final project summaries follow along with a list of their peer-reviewed publications during their PBF Fellowship.

**Zoulfia Allakhverdi, PhD****Mentor:** Guy Delespesse, MD, PhD**Institution:** CHUM Research Center, Montreal, Quebec**Project title:** The role of epithelial cell-derived cytokines in allergic diseases**PBF Project Summary, May 2011:**

While I was supported by a Parker B. Francis Fellowship we have reported that blood CD34<sup>+</sup> hemopoietic cells, in addition to being progenitors of major effectors of allergic inflammation such as eosinophils, basophils and mast cells, also display a robust proinflammatory activity in response to epithelial cell-derived cytokines and act themselves as effector cells (Allakhverdi Z. et al *J. Allergy Clin. Immunol.* 2009 Feb; 123(2): 472-8). This important study identified progenitor cells as innate essential source of Th2 cytokines. This novel concept of dual function of progenitor cells as precursor cells and proinflammatory cells has important therapeutic implications: current antiallergic treatments may adversely affect the proinflammatory activity of progenitor cells. Indeed, we have demonstrated that neutralization of IL-5 does not affect the ability of progenitor cells to respond to epithelial cell-derived cytokines and to produce high levels of Th2 cytokines implicated in the pathogenesis of asthma and allergic diseases, thus possibly explaining the clinical inefficacy of anti-IL-5 treatment. Moreover, we have demonstrated that glucocorticoids, commonly used in anti-asthma drugs, significantly upregulate TSLP receptor on mast cells and their early progenitors and this upregulation was linked to increased response of mast cells to epithelial cell-derived cytokines for the production of Th2 cytokines.

Proinflammatory activity of CD34 progenitor cells prompted us to further investigate the “bone marrow hypothesis” of allergic diseases: involvement of the bone marrow and hemopoietic progenitors in allergic disease and tissue inflammatory responses. In collaboration with AllerGen investigators we are currently examining whether the production of TSLP and the responsiveness to TSLP are different in normal control and in allergic asthmatic subjects. These studies have the potential to elucidate the mechanisms involved in the systemic aspects of allergic diseases: “how the nose speaks to the bone marrow”.

Presently, I would like to further explore our unexpected finding of proinflammatory activity of hemopoietic stem/progenitor cells in application to cystic fibrosis (CF) and severe asthma and to explore the implication of progenitor cells in the pathogenesis of these diseases. Patients with CF experience declining pulmonary function related to chronic airway inflammation that promotes neutrophil influx into the airways. I am planning to study the molecular mechanisms by which airway epithelium may contribute to abnormal lung inflammation in CF and the mechanisms regulating neutrophilic inflammation in response to infectious agents.

I am very grateful to Francis Family Foundation for giving me the PBF Fellowship and making possible my contribution to the advancement of basic science of allergic/pulmonary diseases.

**Publications during PBF Fellowship:**

1. **Allakhverdi Z.**, M.R. Comeau, D.E. Smith, D. Toy, L.M. Endam, M. Desrosiers, Y.J. Liu, K.J. Howie, J.A. Denburg, G.M. Gauvreau, and G. Delespesse. CD34<sup>+</sup> Hemopoietic Progenitor Cells are Potent Effectors of Allergic Inflammation. *J. Allergy Clin. Immunol.* 2009;123:472-478.
2. **Allakhverdi Z.**, M.R. Comeau, H.K. Jessup, and G. Delespesse. TSLP as a mediator of crosstalk between bronchial smooth muscles and mast cells. *J. Allergy Clin. Immunol.* 2009;123:958-960.
3. **Allakhverdi Z.**, M.R. Comeau, and G. Delespesse. Dexamethasone regulation of TSLP receptor expression on mast cells and their precursors. *J. Allergy Clin. Immunol.* 2010, Nov17. doi:10.1016/j.jaci.2010.09.028

**Xiaoyong Bao, PhD****Mentor:** Roberto P. Garofalo, MD**Institution:** University of Texas Medical Branch at Galveston**Project Title:** Innate immune response to human metapneumovirus**PBF Project Summary, May 2011:**

Respiratory virus infections, including hMPV and RSV infections, have been increasingly recognized as an important cause of serious respiratory illness in infants, young children, and immune compromised patients. Innate immune responses act as a first line of defense against viral infection, and also modify adaptive immune responses. Therefore, understanding the host-virus interaction at the innate immune response stage is extremely important, as revealing the role of viral proteins or functional amino acids in host defense and viral evasion will provide important insight for vaccine development and therapeutic strategies, the long-term goals of our research. The Parker B. Francis Fellowship allows me to collect lots of information on how glycoprotein of hMPV regulates the host defense system, using a combination of molecular virology, cellular biology and protein chemistry techniques. The established systems in the period of training will be continuously used to study the functions of other viral protein(s) and emerging roles non-small coding RNAs in mediating the interaction between hMPV/RSV and the host. We hope these studies will eventually lead to the discovery of therapeutic strategies and development of novel vaccine candidate(s) with the best balance between attenuation and effective immunogenicity.

**Publications during PBF Fellowship:**

1. **Bao X**, Sinha M, Hong C, Luxon B, Garofalo RP, and Casola A. Identification of human metapneumovirus-induced gene networks in airway epithelial cells by cDNA microarrays. *Virology* 2008, 368:91-101.
2. Liao S, **Bao X**, Liu T., Hong C, Garofalo RP and Casola A. Role of RIG-I in human metapneumovirus-induced cellular signaling. *J of Gen Virol* 2008, 89:1978-86.
3. **Bao X**, Kolli D, Liu T., Shan Y, Garofalo RP and Casola A. Human metapneumovirus small hydrophobic protein inhibits NF- $\kappa$ B transcriptional activity. *J Virol.* 2008, 82:8824-29.
4. **Bao X**, Liu T, Shan Y, Li K, Garofalo RP, and Casola A. Human metapneumovirus glycoprotein G inhibits innate immune responses. *PLoS Pathog.* 2008 May 30;4(5):e1000077.
5. **Bao X**, Indukuri H, Liu T, Liao S, Tian B, Brasier AR, Garofalo RP and Casola A. IKK $\epsilon$  modulates RSV-induced NF- $\kappa$ B-dependent gene transcription. *Viol* 2010; 408:224-31.
6. Kolli D\*, **Bao X\***, Liu T, Hong C, Garofalo R.P. and Casola A. Human metapneumovirus glycoprotein G modulates Toll-like receptor-4 signaling in monocyte-derived dendritic cells (\*, equal contribution, *J of Immun*, 2011 accepted).

## Natalie N. Bauer, PhD

**Mentor:** Ivan F. McMurtry, PhD

**Institution:** University of South Alabama

**Project Title:** Pulmonary microvascular endothelial response to acute hypoxia: RhoA/Rho kinase signaling and microparticles.



### **PBF Project Summary, May 2011:**

The PBF fellowship has allowed me to launch multiple projects that will ultimately benefit patients with lung disease. My current work is focused on understanding whether small vesicles, circulating in the blood can give physicians and scientists valuable information regarding the status of the pulmonary vessels. In patients with pulmonary arterial hypertension it is currently impossible to determine the exact injury to the pulmonary vessels in order to treat patients. Further, these vesicles, called microparticles, may contribute directly to the vascular injury. Thus, by knowing where the lung-derived microparticles are coming from and what they are doing we may be better able to assess a patient's well-being and treat them more effectively.

Our findings are that microparticles released from pulmonary microvascular endothelial cells activated with different stimuli, including low oxygen and cigarette smoke, are released by different mechanisms from the cell and carry on their surface unique markers. Further, when we examined microparticles from the blood of an animal model of pulmonary arterial hypertension we have found that the types of microparticles change depending on the progression of the animal's disease. Generating data like these will ultimately allow physicians to analyze a single blood sample and determine the extent of injury to pulmonary blood vessels and hopefully lead to new treatments capable of inhibiting the release of harmful microparticles.

### **Publications during PBF Fellowship:**

1. King J, Syklawer E, Chen H, Resmondo J, McDonald F, Stevens T, Shevde L, Ofori-Acquah S, Moore T, and **Bauer N**. Lung endothelial cells express ALCAM on released exosomes/microparticles. *Microsc Microanal* 2008; 14(Suppl 2).
2. King J, Agarwal S, Syklawer E, Prasain N, Chen H, Resmondo J, McDonald F, **Bauer N**, Alvarez D, Wu S, Stevens T, Shevde L, Moore T, and Townsley M. Quantum dots-Utilization in TEM. *Microsc Microanal* 2008; 14(Suppl 2).
3. **Bauer N**, Rai J, Chen H, Harris L, Shevde ., Moore T, and King J. Microparticles/Exosomes: Isolation and TEM Analysis. *Microscopy Today* 2009;17(2):42-45.

**Kevin J. Cummings, PhD****Mentor:** Eugene E. Nattie, MD**Institution:** Dartmouth Medical School**Project Title:** 5-HT disruptions and reflex control of heart rate: implications for SIDS**PBF Project Summary, May 2011:**

Severe hypoxia is common in premature infants – including those with lung pathology – and can manifest in the form of neonatal asphyxia, acute life-threatening events or SIDS. The ability of infants to survive severe hypoxia depends on autoresuscitation, a well-conserved process in mammals whereby gasping helps reverse the inhibitory effects of hypoxia on breathing and heart rate. While reduced preparations have helped identify the important neural substrates underpinning the generation of gasping, reducing mortality in infants susceptible to severe hypoxia (including those with lung pathology) will ultimately rely on the development of new animal models and methodologies that can identify key components of the intact system required for successful autoresuscitation.

**Key ideas and discoveries made possible by the PBF Fellowship Program**

- The PBF Fellowship has supported my research that utilizes a unique system to measure ventilation and heart rate in unanesthetized, neonatal rodents with brainstem 5-HT deficiency.
- Neonatal rodents deficient in 5-HT neurons exhibit apnea, bradycardia and profound defects in autoresuscitation. As a consequence, these animals can withstand significantly fewer episodes of hypoxia compared to control littermates. A compelling aspect of the phenotype is its emergence at a critical period, ~postnatal day (P)7-10, and its disappearance by ~P12.
- Systemic application of low doses of caffeine to Pet-1<sup>-/-</sup> mice restores autoresuscitation and extends the number of episodes of severe hypoxia that they can tolerate.
- Caffeine offers a potentially exciting and effective prophylactic for newborns susceptible to neonatal asphyxia, acute life-threatening events or SIDS. This may include newborns with overt lung pathology. As a new investigator at the University of Missouri, I will be investigating the mechanisms underpinning the protective effects of caffeine.

**Publications during PBF Fellowship:**

1. Wilson RJA, **Cummings KJ**. Pituitary adenylate cyclase-activating peptide: vital for neonatal survival and the neuronal control of breathing. *Resp Physiol Neurobiol* 2008; 164:168-78.
2. **Cummings KJ**, Commons K, Fan K, Li A, Nattie EE. Severe spontaneous bradycardia associated with Respiratory disruptions in rat pups with fewer brainstem 5-HT neurons. *Am J Physiol Reg Integr Comp Physiol* 2009; 296(6):R1783-96
3. **Cummings KJ**, Frappell P. The breath-to-breath hypercapnic response in neonatal rats: temperature dependency of the chemoreflexes and potential implications for breathing stability. *Am J Physiol Reg Integr Comp Physiol* 2009; 297):R124-134.
4. **Cummings KJ**, Li A, Deneris E, Nattie EE. Bradycardia in serotonin deficient Pet-1<sup>-/-</sup> mice: influence of respiratory dysfunction and hyperthermia over the first two postnatal weeks. *Am J Physiol Reg Integr Comp* 1010; 298:R1333-1342.
5. **Cummings KJ**, Li A, Nattie EE. Brainstem serotonin deficiency in the neonatal period: autonomic dysfunction during mild cold stress. *J Physiol* 2011; 589:2055-264.
6. **Cummings KJ**, Commons KG, Hewitt JC, Daubenspeck JA, Li A, Kinney HC, Nattie EE. Failed heart rate recovery at a critical age in 5-HT-deficient mice exposed to episodic severe hypoxia: Implications for SIDS. *J Appl Physiol* 2011; *in press*.

## Charles S. Dela Cruz, MD, PhD

**Mentor:** Jack A. Elias, MD

**Institution:** Yale University

**Project Title:** The role of cigarette smoke exposure and respiratory infections in emphysema



### PBF Project Summary, May 2011:

Tobacco use has been known for decades to be harmful for the lungs causing respiratory pathologies such as chronic obstructive pulmonary diseases (COPD), an entity that can be characterized by bronchitis and emphysema, one of the leading causes of death in the world. The goal of the funded PBF Fellowship grant is to investigate the interaction between cigarette smoke (CS) and respiratory virus infection in the pathogenesis of COPD and identify novel therapeutic targets for this respiratory disease. It has been long thought that the frequent respiratory infections in COPD patients are due to their depressed immune function. Frequent acute COPD flares correlate with increased rate of disease progression and more loss of lung function in COPD, especially if it is due to viral infections. Our findings as the result of the PBF studies revealed that CS-exposed hosts have an over exaggerated immune reaction to infections.

We have identified novel cellular and molecular pathways to explain the exaggerated inflammatory, injury and repair responses to respiratory viral infections in hosts who are exposed to cigarette smoke. We find that CS exposure has an impressive ability to regulate the innate immune response in the lung after influenza virus and respiratory syncytial virus (RSV) infection. CS enhances the inflammation, alveolar destruction and airway fibrosis caused by influenza virus and RSV. These effects are mediated by the antiviral innate immune pathways that involves type I interferon and RIG-like helicases. We have identified that the cytokine interleukin-15 (IL-15) is important in the synergistic interaction between CS exposure and virus infection. Moreover, we have also identified that members of the chitinase and chitinase-like proteins are upregulated in hosts exposed to both CS and viruses and that these proteins are important in the pathogenesis of COPD. The novel findings as a result of the PBF funded studies helped identify new molecular targets that could be pursued in future therapeutics for COPD, a disease that is in much need of new treatment options for our patients. Moreover, it highlights the importance of preventing respiratory infections, including vaccination in our patients with COPD or patients with smoking exposure history.

### Publications during PBF Fellowship:

1. Kang MJ, Lee CG, **Dela Cruz CS**, Enelow R and Elias JA. Cigarette Smoke Selectively Enhances Viral PAMP and Virus-Induced Innate Immune Responses and Emphysematous Tissue Destruction in the Murine Lung. *J Clin Invest.* 2009;118:2771-2784.
2. **Dela Cruz CS**, Viswanathan SR, El-Guindy AS, Shedd D, Miller G. Complex N-linked glycans on Asn-89 of Kaposi sarcoma herpes virus-encoded interleukin-6 mediate optimal function by affecting cytokine protein conformation. *J Biol Chem.* 2009; 284(43):29269-82.
3. **Dela Cruz CS** and Matthay RA. Role of obesity in cardiomyopathy and pulmonary hypertension. *Clin Chest Med.* 2009, 30(3): 509-523.
4. **Dela Cruz CS**, Kang MJ, Cho WK, Lee CG. 2010. Transgenic Modeling of Cytokine Polarization in the Lung, *Immunology*, 132(1):9-17.
5. Lee CG, Da Silva C, **Dela Cruz CS**, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA. Role of chitin, chitinase/chitinase-like proteins (C/CLP) in inflammation, tissue remodeling and injury, *Annual Review of Physiology*, 2010;73:479-501.
6. Matsuura H, Hartl D, Kang MJ, **Dela Cruz CS**, Koller B, Chupp GL, Homer RJ, Lee CG and Elias JA. Role of Breast Regression Protein (BRP) 39 in the Pathogenesis of Cigarette Smoke-Induced Inflammation and Emphysema, *AJRCMB*; 2010, July 23.
7. Ma B, **Dela Cruz CS**, Hartl D, Kang MJ, Takyar S, Homer RJ, Lee CG, Elias JA. RIG-Like Helicase Innate Immunity Inhibits VEGF Tissue Responses via A Type Interferon-Dependent Mechanism. *Am J Respir Crit Care Med.* 2011;183(10):1322-35.

## Elena A. Goncharova, PhD

**Mentor:** Reynold A. Panettieri, Jr., MD (1989 PBF Fellow)

**Institution:** University of Pennsylvania School of Medicine

**Project Title:** RhoA GTPase modulates cell proliferation in lymphangio-leiomyomatosis (LAM)



### PBF Project Summary, May 2011:

LAM is the rare lung disease, affecting primarily women of childbearing age, which is characterized by abnormal growth of smooth muscle-like cells within the lung caused by dysfunction of tumor suppressor protein TSC2. Previously, we demonstrated that TSC2 dysfunction promotes LAM cell growth via activation of mTOR complex 1 (mTORC1), which established mTORC1 inhibitor rapamycin as promising therapeutic approach to inhibit LAM cell growth. Recent clinical studies show that rapamycin improves pulmonary functions and reduces the size of angiomyolipomas in LAM-TS patients during treatment time. The cessation of rapamycin therapy, however, resulted in the return to diminished pulmonary function and AML tumors to that observed prior to rapamycin treatment [*N Engl J Med* 2008, 358:140-151; 2011, 364:1595-1606] suggesting that alternative or combinational therapies are needed to treat LAM. We have shown that TSC2 dysfunction also leads to activation of small protein RhoA GTPase that contributes to increased growth and survival of TSC2-null and LAM cells and is not inhibited by rapamycin. We also demonstrated that increased RhoA activity is due to activation of rapamycin-insensitive mTORC2. Importantly, we found that combined targeting of mTORC2-RhoA by simvastatin and mTORC1 by rapamycin is more effective than either drug alone to inhibit growth of human LAM cells. Further, our data show that simvastatin induces cell death in TSC2-null tumors in mice and, when given in combination with rapamycin, blocks tumor growth and prevents tumor re-growth [*Mol Cell Biol* 2011; 31(12):2484-98]. Taken together, our findings indicate that, in addition to rapamycin-sensitive mTORC1, TSC2 dysfunction promotes activation of rapamycin-insensitive mTORC2-RhoA in TSC2-null and LAM cells. We also show that combined inhibition of mTORC1 by rapamycin and RhoA by simvastatin have benefits compared to single rapamycin or simvastatin treatments in inhibiting LAM cell and TSC2-null tumor growth, inducing cell death and preventing TSC2-null tumor re-growth after treatment termination. Our findings suggest that mTORC2-RhoA may serve as potential therapeutic target for combinational therapy of LAM.

### Publications during PBF Fellowship:

1. Krymskaya VP and Goncharova EA. PI3K/mTORC1 activation in hamartoma syndromes: therapeutic prospects. *Cell Cycle* 2009;8(3):403-413.
2. Damera G, Fogle H, Lim P, Goncharova E, Zhao H, Banerjee A, Tliba O, Krymskaya V, Panettieri R, Jr. Vitamin D inhibits human airway smooth muscle growth through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol* 2009;158(6):1429-1441.
3. Goncharova EA, Goncharov DA, Damera G, Tliba O, Amrani Y, Panettieri RA, Krymskaya VP. STAT3 is required for abnormal proliferation and survival of TSC2-deficient cells: relevance to pulmonary LAM. *Mol Pharmacol* 2009;76(4):766-777.
4. Goncharova EA, Lim PN, Chisolm A, Fogle HW III, Taylor JH, Goncharov DA, Eszterhas A, Panettieri RA Jr., Krymskaya VP. Interferons modulate mitogen-induced protein synthesis in airway smooth muscle (ASM). *Am J Physiol Lung Cell Mol Physiol* 2010; 299:L25-L35.
5. Krymskaya VP, Snow J, Cesarone G, Khavin I, Goncharov D, Lim P, Veasey S, Ihida-Stansbury K, Jones P, Goncharova EA. mTOR is required for pulmonary arterial vascular smooth muscle cell proliferation under chronic hypoxia. *FASEB J* 2011; [Epub ahead of print]
6. Goncharova EA, Goncharov DA, Li H, Pimtong W, Lu S, Khavin I, Krymskaya VP. mTORC2 is required for proliferation and survival of TSC2-null cells. *Mol Cell Biol* 2011; [Epub ahead of print]

**Steven K. Huang, MD****Mentor:** Marc Peters-Golden, MD**Institution:** University of Michigan**Project Title:** Epigenetic regulation of the E prostanoid 2 receptor gene in fibrotic lung fibroblasts**PBF Project Summary, May 2011:**

Idiopathic pulmonary fibrosis is a devastating disease characterized by excessive scarring in the lungs. It affects predominantly the elderly and results in significant morbidity and mortality, with death occurring at a median of 3-5 years from the time of diagnosis. There are currently no effective treatments. Fibroblasts are the main effector cells that accumulate in the lung and are responsible for the excessive scarring associated with fibrosis. It has previously been shown that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a lipid mediator produced by many cells in the lung, helps protect against fibrosis by inhibiting fibroblast functions. We previously observed that fibroblasts cultured from the lungs of IPF patients and from mouse models of fibrosis exhibited resistance to PGE<sub>2</sub>, in part due to decreased expression of its receptor, E prostanoid 2 (EP2). We

hypothesized that epigenetic changes – namely DNA methylation and histone modifications – are responsible for the decreased EP2 expression. We have now discovered that DNA methylation changes are indeed responsible for the diminished EP2 expression. IPF cells and cells from mouse models of fibrosis treated with DNA methylation inhibitors exhibited higher levels of EP2 expression, which restored the ability of PGE<sub>2</sub> to inhibit these cells. These findings delineate a novel mechanism by which fibroblasts from fibrotic lung establish and maintain a profibrotic state via epigenetic modifications. Drugs that inhibit methylation, of which many are already used for cancer, may serve as a novel therapeutic agent in this deadly disease.

Huang SK, Fisher AS, Scruggs AM, White ES, Hogaboam CM, Richardson BC, Peters-Golden M. Hypermethylation of *PTGER2* Confers Prostaglandin E<sub>2</sub> Resistance in Fibrotic Fibroblasts from Humans and Mice. *Am J Pathol.* 2010 Nov;177(5):2245-55.

**Publications during PBF Fellowship:**

1. **Huang SK**, Wettlaufer SH, Chung J, Peters-Golden M. Prostaglandin E<sub>2</sub> Inhibits Specific Lung Fibroblast Functions Via Selective Actions of PKA and Epac-1. *Am J Respir Cell Mol Biol.* 2008 Oct;39(4):482-9.
2. Chung J, Serezani CH, **Huang SK**, Stern JN, Keskin DB, Jagirdar R, Brock TG, Aronoff DM, Peters-Golden M. Rap1 activation is required for Fc gamma receptor-dependent phagocytosis. *J Immunol.* 2008 Oct 15;181(8):5501-9.
3. Hao Y, Senn T, S Opp J, Young VB, Thiele T, Srinivas G, **Huang SK**, Aronoff DM. Lethal toxin is a critical determinant of rapid mortality in rodent models of *Clostridium sordellii* endometritis. *Anaerobe.* 2010 Apr;16(2):155-60.
4. Sagana RL, Yan M, Cornett AM, Tsui JL, Stephenson DA, **Huang SK**, Moore BB, Ballinger MN, Melonakos J, Kontos CD, Aronoff DM, Peters-Golden M, White ES. Phosphatase and tensin homologue on chromosome ten (PTEN) directs prostaglandin E<sub>2</sub>-mediated fibroblast responses via regulation of E prostanoid 2 receptor expression. *J Biol Chem.* 2009 Nov 20;284(47):32264-71.
5. **Huang SK**, Myers JL, Flaherty KR. Diagnosing idiopathic interstitial pneumonia: utility of surgical lung biopsy. *Eur Resp Monograph.* 2009;46:24-35.
6. **Huang SK**, White ES, Wettlaufer SH, Grifka H, Hogaboam CM, Thannickal VJ, Horowitz JC, Peters-Golden M. Prostaglandin E<sub>2</sub> induces fibroblast apoptosis by modulating multiple survival pathways. *FASEB J.* 2009 Dec;23(12):4317-26.
7. Bauman KA, Wettlaufer SH, Okunishi K, Vannella KM, Stoolman JS, **Huang SK**, Courey AJ, White ES, Hogaboam CM, Simon RH, Toews GB, Sisson TH, Moore BB, Peters-Golden M. The antifibrotic effects of plasminogen activation occur via prostaglandin E<sub>2</sub> synthesis in humans and mice. *J Clin Invest.* 2010 Jun 1;120(6):1950-60.
8. **Huang SK**, Fisher AS, Scruggs AM, White ES, Hogaboam CM, Richardson BC, Peters-Golden M. Hypermethylation of *PTGER2* Confers Prostaglandin E<sub>2</sub> Resistance in Fibrotic Fibroblasts from Humans and Mice. *Am J Pathol.* 2010 Nov;177(5):2245-55.

## Guillaume Lenormand, PhD

**Mentor:** Jeffrey J. Fredberg, PhD

**Institution:** Harvard School of Public Health

**Project Title:** Why is the asthmatic airway refractory to deep inspirations?



### PBF Project Summary, May 2011:

My study aims at understanding the mechanics of the airway smooth muscle (ASM), and identifying the physical principles underlying ASM dynamics. My work led to two unexpected findings.

The first unexpected finding is that the internal time scale of the muscle is set not by the internal viscosity of the muscle itself, but rather by the amplitude of the external forcing. As a consequence, the dynamics of the airway is inconsistent with any traditional notion of viscoelasticity or viscous stress, as very often used in the literature to model smooth muscle dynamics. As a consequence of strain rate amplitude and power law rheology, the mechanical properties of the smooth muscle are found to depend very little on the frequency of the excitation, but mostly on the strain of excitation. Going back to a deep inspiration and its effect on airway compliance, these data imply that only strain matters, not

frequency. In other words, it does not matter how fast you go, it only matters how far you go. This work was published in *Physical Review Letters* (Oliver et al., *Phys. Rev. Lett.*, 2010).

The second finding is that cytoskeletal dynamics and many cellular functions, such as fluidization induced by large stretch (similar to a deep inspiration) are modulated by water dynamics. It has previously been shown that protein dynamics were controlled to the hydration shell. I first investigated water kind, replacing intra cellular water with heavy water, and have shown for the first time that water is implied in protein dynamics *in vivo* as well. This finding could have interesting implication in asthma and how a deep inspiration modifies cytoskeletal dynamics. This work has been recently accepted for publication in *Physical Review E* (Lenormand et al., *Phys. Rev. E*, 2011). In addition to water kind, I looked, with Dr. Zhou, at the importance of water quantity. By changing the amount of water within the cell, we were able to identify a range of dynamics that has previously only been seen in inert soft matter as colloidal systems. This interesting work further highlights the importance of water within the living cell, and was published in *PNAS* (Zhou et al., *PNAS*, 2009).

### Publications during PBF Fellowship:

1. Krishnan R., Park C.Y., Mead J., Jaspers R.A., Trepap X., Lenormand G., Butler J.P., Fredberg J.J., Airway smooth muscle and bronchospasm: fluctuating, fluidizing, freezing, *Respiratory Physiology & Neurobiology*, 2008; 30;163(1-3):17-24.
2. Trepap X., Lenormand G., Fredberg J.J., Universality in cell mechanics, *Soft Matter*, 2008; 4(9):1750-1759.
3. Mott A., Lenormand G., Costales J, Fredberg J.J., Burleigh B.A., Modulation of host cell mechanics by *Trypanosoma cruzi*, *J Cell Physiol.*, 2009; 218(2):315-22.
4. Krishnan R., Park C.Y., Mead J., Jaspers R.T., Trepap X., Lenormand G., Tambe D., Butler J.P., Fredberg J.J., Reinforcement vs fluidization in cytoskeletal mechanoprotection, *PLoS ONE*, 2009; 4(5):e5486.
5. Zhou E.H., Trepap X., Park C.Y., Lenormand G., Oliver M.N., Mijailovich S.M., Hardin C., Weitz D.A., Butler J.P., Fredberg J.J., Universal Behavior of the Osmotically Compressed Cell and its Analogy to the Colloidal Glass Transition, *Proc Natl Acad Sci U S A.*, 2009; 106(26):10632-7.
6. DiPaolo B.C., Lenormand G., Fredberg J.J., Margulies S.S., Stretch magnitude and frequency dependent actin cytoskeleton remodeling in primary alveolar epithelia, *Am. J. Physiol.*, 2010; 299(2):C345-53.

**Guillaume Lenormand Publications (Continued)**

7. Mechanosensing of substrate thickness. Lin Y.C., Tambe D.T., Park C.Y., Wasserman M.R., Trepats X., Krishnan R., Lenormand G., Fredberg J.J., Butler J.P., *Phys Rev E Stat Nonlin Soft Matter Phys.* 2010;82(4 Pt 1):041918.
8. Remodeling of integrated contractile tissues and its dependence on strain-rate amplitude. Oliver M., Kováts T., Mijailovich S.M., Butler J.P., Fredberg J.J., Lenormand G., *Phys Rev Lett.*, 2010; Oct 8;105(15):158102.
9. Dynamics of the cytoskeleton: How much does water matter?, Lenormand G., Millet E., Park C.Y., Cardin C.C., Butler J.P., Moldovan N.I., Fredberg J.J. 2011; *Phys Rev E Stat Nonlin Soft Matter Phys.*, In press.
10. Therapeutic ultrasound perturbs cytoskeleton dynamics, Mizrahi N., Zhou E.H., Lenormand G., Krishnan R., Weihs D., Butler J.P., Weitz D.A., Fredberg J.J., Kimmel E., 2011, *J. App. Physiol.* In press.

## Peter M. MacFarlane, PhD

**Mentor:** Gordon S. Mitchell, PhD

**Institution:** University of Wisconsin

**Project Title:** Serotonergic modulation of spinal NADPH oxidase is necessary and sufficient for intermittent hypoxia-induced phrenic long-term facilitation



### PBF Project Summary, May 2011:

The Parker B Francis Fellowship supported my research to investigate neural mechanisms of respiratory plasticity and the signaling molecules involved including ROS (reactive oxygen species), NO (nitric oxide), serotonin and glycolytic metabolism. These data will provide insight into how the respiratory control system adapts in response to environmental, physiological or pathological conditions. Such compensatory plasticity may enable the respiratory control system to respond appropriately to AIH (acute intermittent hypoxia), promoting mechanisms that prevent its recurrence such as been hypothesized in OSA patients. These data might also contributed to the development of novel therapeutic strategies in the treatment of potentially fatal or debilitating respiratory disorders such as COPD and respiratory insufficiency following spinal cord injury or amyotrophic lateral sclerosis. The overall outcome from my studies has been to provide insight into harnessing neural mechanisms of respiratory plasticity to promote respiratory recovery during conditions associated with compromised respiratory function. The laboratory currently collaborates with members of the Rehab Institute of Chicago and the Division of Physical Therapy at Emory University for the use of intermittent hypoxia in spinally injured patients. So, interestingly, these data are becoming relevant not just for spinal respiratory-related motor nuclei, but thoracic and lumbar regions as well.

In my new role as Assistant Professor in Pediatrics, I am developing a laboratory in which I will be able to investigate the postnatal development of respiratory control mechanisms. Intermittent hypoxia is commonly associated with apnoea of prematurity and I am committed to a research career path aimed at understanding the progression of apnoea and its effects on postnatal development. I am also interested in other factors experienced during prematurity including airway (and neural) inflammation and the long-term effects of supplemental oxygen on respiratory control mechanisms. I also hope to develop some insight into the effects of these early life experiences on the control of metabolically sensitive genes that are key to “normal” postnatal development of lung and respiratory neural control mechanisms. I hope to develop a laboratory capable pioneering insight into new treatments to improve the care and well-being of premature infants.

### Publications during PBF Fellowship:

1. **MacFarlane PM**, Vinit S, Mitchell GS. Serotonin 2A and 2B receptor-induced phrenic motor facilitation: differential requirement for spinal NADPH oxidase activity. *Neurosci* 2011; 178:45-55.
2. Dale-Nagle EA, Hoffman MS, **MacFarlane PM**, Satriotomo I, Lovett-Barr MR, Vinit S, Mitchell GS. Spinal plasticity following intermittent hypoxia: implications for spinal injury. *Ann NY Acad Sci* 2010; 1198:252-259.
3. Dale-Nagle EA, Hoffman MS, **MacFarlane PM**, Mitchell GS. Multiple pathways to long-lasting phrenic motor facilitation. *Adv Exp Med Biol.* 2010; 669:225-230.
4. Baker-Herman TL, Bavis RW, Dahlberg JM, Mitchell AZ, Wilkerson JE, Golder FJ, **Macfarlane PM**, Watters JJ, Behan M, Mitchell GS. Differential expression of respiratory long-term facilitation among inbred rat strains. *Respir Physiol Neurobiol* 2010; 170(3):260-267.
5. **Macfarlane PM**, Satriotomo I, Windelborn JA, Mitchell GS. NADPH oxidase activity is necessary for acute intermittent hypoxia-induced phrenic long-term facilitation. *J Physiol* 2009; 587(9): 1931-1942.
6. **MacFarlane PM**, Mitchell GS. Episodic spinal serotonin receptor activation elicits long-lasting phrenic motor facilitation by an NADPH oxidase-dependent mechanism. *J Physiol* 2009; 587(Pt 22):5469-5481.
7. **MacFarlane PM**, Wilkerson JE, Lovett-Barr MR, Mitchell GS. Reactive oxygen species and respiratory plasticity following intermittent hypoxia. *Respir Physiol Neurobiol* 2008; 164(1-2):263-271.

**Brian J. Mitchell, PhD****Mentor:** Robert D. Goldman, PhD**Institution:** The Salk Institute, La Jolla, CA**Project Title:** The generation and maintenance of directed mucus flow**PBF Project Summary, May 2011:**

Cilia are motile hair-like extensions of cells that line the respiratory tract and beat in a directed manner to transport mucus, thereby clearing the lungs of bacteria and debris. Respiratory disease is often associated with genetic or environmental defects in the cilia formation or the orientation that they beat. We have used the skin of frog embryos, which is remarkably similar to the respiratory track to address the molecular mechanisms responsible for generating and orienting cilia. We discovered the polarity signals which originate from neighboring cells to communicate polarity information to the cells with cilia. Furthermore we identified the cellular mechanisms that cells employ to interpret these signals to drive the orientation of individual cilia. The loss of cilia often accompanies respiratory distress. We have developed an experimental paradigm that allows us to deciliate frog skin and then study the ability of cilia to regrow. Furthermore we have identified a molecular pathway that impinges on this regrowth. The hope is that by elucidating these pathways we will identify targets for drug discovery that can be used to stimulate both regrowth and the stabilization of cilia polarity during respiratory stress. The final Aim of this grant was to perform a screen to identify novel genes involved in the process of cilia formation and polarization. This screen has been very successful and the characterization of these novel genes will continue to provide novel directions for our research. Currently, we are characterizing one gene identified from this screen which is involved in the process of centriole duplication. Centrioles are cellular structures that are critical during cell division and defects in centriole duplication correlate significantly with various forms of cancer. Centrioles are also critical for the formation of cilia and thus are prevalent in high numbers in both respiratory cells and our frog skin model. We identified the first molecular component of the pathway that drives centriole formation in ciliated cells. This protein localizes to a molecularly uncharacterized cellular structure termed the dueterosome that was previously identified only by Electron Microscopic studies. Manipulation of this structure and pathway has never been achieved and we anticipate that understanding the process of centriole duplication will again provide targets for drug discovery that may lead to treatments for fostering regeneration of cilia after respiratory stress.

**Publications during PBF Fellowship:**

1. **Mitchell, B.J.**, Stubbs, J., Huisman, F., Taborek, P., Yu, C., Kintner, C. The PCP pathway instructs the planar orientation of ciliated cells in the *Xenopus* larval skin. *Current Biology*. 2009 Jun 9; 19(11): 924-9.
2. Dammerman A., Pemble H., **Mitchell B.**, Kintner C., Oegema K. The Hydrolethalus syndrome protein HYLS-1 links core centriole structure to cilia formation. *Genes and Development*. 2009 Sep 1; 23(17): 2046-59.

## Laura M. Palermo, PhD

**Mentor:** Anne Moscona, MD

**Institution:** Weill Cornell Medical College

**Project Title:** Screening of antiviral compounds and characterization of their effect on paramyxovirus infection in the respiratory tract.



### PBF Project Summary, May 2011:

Parainfluenza virus 3 (HPIV3) infection is the predominant cause of croup in young infants and a common agent of bronchiolitis and pneumonia. These are respiratory diseases that severely affect young infants and children worldwide, especially babies born prematurely or with congenital abnormalities. In addition, parainfluenza viruses are emerging as important causes of life-threatening pneumonia in immunocompromised patients of all ages, for example adults with leukemia. There are no vaccines yet for the parainfluenza viruses, and any vaccine is unlikely to protect the very youngest infants and the immunocompromised. There are no weapons against the parainfluenza viruses. The principal obstacles to preventing and treating croup, bronchiolitis and other diseases caused by parainfluenza in children are caused by the gaps in

our understanding of fundamental processes of paramyxovirus biology in the lung. Several features of the viral life cycle make paramyxoviruses vulnerable to attack. They enter the lung cell by binding to a receptor molecule and then fusing their outer layer with the lung cell membrane to gain admittance into the cell. Both binding and fusion are critical for infection. Interference with these essential events would suffice to prevent disease. In our research we identified compounds that block the early steps of infection and we characterized their mechanism of action. Some of our compounds mimic the lung cell, stimulate the virus to fuse at the wrong time and place and reduce virus infectivity. The efficacy of these antiviral compounds was tested in a model of human respiratory system that works as a lung. In a second approach we attached cholesterol to some of our antivirals, this method made these compounds more effective against the virus, which suggests that they are promising candidates for the prevention or therapy of infection with paramyxoviruses. Our work gives us a better understanding for how virus-receptor interaction leads to viral entry into the respiratory tract and indicates that our compounds would be clinically useful in reducing the duration of the disease, and halt the spread of an epidemic.

### Publications during PBF Fellowship:

1. **Palermo, LM.**, Porotto, M., Yokoyama, C., Palmer, SG., Greengard, O. Mungall, BA., Niewiesk, S., Moscona, A. (2009). "Human parainfluenza virus infection of the airway epithelium: the viral hemagglutinin-neuraminidase regulates F-triggering and modulates infectivity." *Journal of Virology*. 83:6900-8.
2. Porotto M, Yokoyama CC, **Palermo LM**, Mungall B, Aljofan M, Cortese R, Pessi A, Moscona A. (2010). "Viral entry inhibitors targeted to the membrane site of action." *Journal of Virology*. 84: 6760-8.
3. Porotto M, Rockx, B., Yokoyama, C. Talekar, A. **Palermo, LM**, Cortese R. Pessi A, Moscona A. (2010). "Inhibition of Nipah Virus Infection In Vivo: Targeting an Early Stage of Paramyxovirus Fusion Activation during Viral Entry." *PLoS Pathogens*. 6(10). e1001168

**Jordi B. Torrelles, PhD****Mentor:** Larry S. Schlesinger, MD**Institution:** Ohio State University**Project Title:** Influence of the human lung hydrolases on *Mycobacterium tuberculosis* infection**PBF Project Summary, May 2011:**

Currently, one person is infected by *Mycobacterium tuberculosis* [*M.tb*, the causing agent of tuberculosis (TB)] every 4 seconds and dies of TB every 18 seconds. A better understanding of the *M.tb* pathway of infection is necessary to develop new therapies against this devastating pathogen. When *M.tb* infection occurs, bacteria is deposited in the deepest spaces within the lung (also called alveolar spaces), where the gas exchange takes place. We discovered that environmental enzymatic activities (called hydrolases) present in the alveolar spaces of the lung are capable of modifying the bacterial surface of *M.tb*. These bacterial cell surface modifications include the removal of critical virulent factors from the surface of *M.tb*. We further demonstrated that these modifications allowed a better control of the *M.tb* infection by the infected host. In this context,

the possibility of altering the human lung environment to our benefit to fight bacterial infections is a new unexplored area of research. Our data provide us with the basis to establish if up- or down-regulation of lung environmental hydrolases could serve as target to prevent *M.tb* infection addressing how the lung environment influences *M.tb* pathogenesis *in vivo* using specific hydrolase deficient mice models or non-toxic inhibitors against specific lung hydrolases. A better understanding of the human lung environment that *M.tb* (and other lung pathogens) encounters during early stages of infection will allow the developing of the necessary tools to manipulate this environment in a way that will benefit the human host by enhancing bacterial killing, minimizing the establishment of chronic infections. In addition, the observed modifications on the *M.tb* bacterial surface produced by the human lung environment in our experiments *in vitro* may mimic those found during *M.tb* infection *in vivo* and thus, opens new avenues in TB diagnostics, therapies and vaccine development. In this context, our results show that as a consequence of the action of the lung environmental hydrolases on the *M.tb* bacterial surface, *M.tb* fragments are released into the lung milieu. These *M.tb* fragments resulted to be modulators of the immune response against infection, and thus their potential use in vaccine development. Importantly, the knowledge gained from our *M.tb* studies can be broadly applied to other significant lung infections. Our current studies with *Pseudomonas aeruginosa* infection in cystic fibrosis patients are evaluating the role of the lung environment hydrolases found in these patients in the establishment of bacterial infections.

**Publications during PBF Fellowship:**

1. **Torrelles JB**, Knaup R, Kolareth A, Slepshkina T, Kaufman TM, Kang P, Hill PJ, Brennan PJ, Chatterjee D, Belisle JT, Musser JM, and Schlesinger LS. Identification of *Mycobacterium tuberculosis* clinical isolates with altered phagocytosis by human macrophages due to a truncated lipoarabinomannan. *J. Biol. Chem.* 2008; 283:31417-31428.
2. Azad AK, **Torrelles JB**, and Schlesinger LS. Mutation in DC-SIGN cytoplasmic triacidic cluster motif attenuates the receptor in both phagocytosis and endocytosis of mannose-containing ligands by human myeloid cells. *J. Leuk. Biol.* 2008; 84:1594-1603.
3. **Torrelles JB**, DesJardin LE, Knaup R, MacNeil J, Kaufman TM, Kutzbach B, Travis R, McCarthy TR, Gurcha SS, Besra GS, Clegg S and Schlesinger LS. Inactivation of the mannosyltransferase PimB reduces surface expression of the cell wall lipoarabinomannan in *Mycobacterium tuberculosis* affecting the rate of macrophage cell death. *Glycobiology* 2009; 19:743-755.
4. Carlson TK, **Torrelles JB**, Smith K, Horlacher T, Crouch E, and Schlesinger LS (2009). Differential recognition of mycobacterial species and their mannosylated cell envelope components by surfactant protein-D. *Glycobiology* 2009; 19:1473-1484.

**Jordi B. Torrelles Publications (Continued)**

5. **Torrelles JB** and Schlesinger LS. Diversity in *Mycobacterium tuberculosis* mannosylated cell wall determinants impacts adaptation to the host. *Tuberculosis* 2010; 90:84-93.
6. Rajaram MVS, Morris JD, Brooks MN, **Torrelles JB**, Azad AK, Schlesinger LS. *Mycobacterium tuberculosis* activates human macrophage PPAR $\gamma$  linking mannose receptor recognition to regulation of immune responses. *J. Immunol.* 2010; 185:929-942.
7. Gangaiah D, Liu Z, Arcos J, Kassem II, Sanad Y, **Torrelles JB** and Rajashekara G. Polyphosphate kinase 2: A novel determinant of stress responses and pathogenesis in *Campylobacter jejuni*. *PLoS One* 2010; 5, pii: e12142, 1-12.
8. Sasindran SJ and **Torrelles JB**. *Mycobacterium tuberculosis* infection and inflammation: What is beneficial for the host and for the bacterium? *Front. Cell. Infect. Microbiol.* 2011; 2:1-16. doi:10.3389/fmicb.2011.00002 [Epub ahead of print].
9. Dwivedi V, Manickam C, Patterson R, Dodson K, Murtaugh M, **Torrelles JB**, Schlesinger LS, Renukaradhya GJ. Cross-protective immunity to porcine reproductive and respiratory syndrome virus by intranasal delivery of a live virus vaccine with a potent adjuvant. *Vaccine* 2011; [Epub ahead of print].
10. Arcos J, Sasindran SJ, Fujiwara N, Turner J, Schlesinger LS, and **Torrelles JB**. Human lung hydrolases delineate *Mycobacterium tuberculosis*-macrophage interactions and the capacity to control infection. *J. Immunol.* 2011 [In press].

**Phuoc T. Tran, MD, PhD****Mentor:** Dean W. Felsher, MD, PhD**Institution:** Stanford University School of Medicine**Project Title:** Investigations on the differential oncogene-dependency of MYC versus K-Ras murine primary lung tumor model systems**PBF Project Summary, May 2011:**

Lung cancer is the number one cancer killer in the United States and the world. Lung cancer causes more cancer related deaths than prostate, breast and colon cancer combined. Lung cancer care is ultimately ineffective because the disease is diagnosed at an advanced stage and because lung cancer is insensitive to current approaches. New treatments targeted against oncogenes, or genes that cause cancer, are dramatically changing the landscape for treatment of lung cancer. "Oncogene-addiction" is a phenomenon whereby lung tumors following oncogene-targeted therapy exhibit dramatic tumor shrinkage. However, only a minority of lung cancers demonstrate addiction to a single oncogene, likely

because multiple oncogenes are active simultaneously. Finding and targeting these additional oncogenes simultaneously could provide large gains in the treatment for lung cancers. Our PBF funded work used mouse lung cancer model systems and suggested that targeting more than one oncogene was more effective for treating lung tumors. We built on this work by using the same mouse lung cancer models to find new oncogene targets for the treatment of lung cancer. One pathway that seemed to be very important for lung tumors was the EGFR/KRAS/BRAF. When we targeted this pathway by multiple means we saw tumor shrinkage in our mouse lung cancer systems. One goal of my PBF proposal was to force lung tumors to exhibit oncogene-addicted behavior after simultaneous targeting of multiple oncogenes.

The second component of my PBF proposal during the independent portion of my proposal was to develop a new mouse model of lung cancer and pulmonary fibrosis (PF). PF is a progressive lung disorder that can end in fatal lung dysfunction from fibrosis or scarring. Current therapies for PF are mainly symptomatic, because the disease cause is poorly defined. During this portion of my PBF award I developed a novel genetically engineered mouse model to facilitate these studies. Future work with this new mouse model has a direct impact on the etiology and possibly treatment for PF and advanced lung cancer.

**Publications during PBF Fellowship:**

1. **Phuoc T. Tran**, Wendy Hara, Zheng Su, H. Jill Lin, Pavan K. Bendapudi, Jeffrey Norton, Nelson Teng, Christopher R. King & Daniel S. Kapp. Intraoperative Radiation Therapy for Locally Advanced and Recurrent Soft Tissue Sarcomas in Adults. *Int J Radiat Oncol Biol Phys.* 72 (2008) 1146-1153.
2. **Phuoc T. Tran**, Alice C. Fan\*, Pavan K. Bendapudi, Shan Koh, Kim Komatsubara, Joy Chen, George Horng, David I. Bellovin, Sylvie Giuriato, Criag S. Wang, Jeffrey A. Whitsett and Dean W. Felsher. Combined Inactivation of MYC and K-ras Oncogenes Reverses Tumorigenesis in Lung Adenocarcinomas and Lymphomas. *PLoS ONE* 3 (2008) e2125.
3. **Phuoc T. Tran** and Dean W. Felsher. The current STATE of Biomarkers for Response to Anti-Angiogenic Therapies. *Cancer Biol Ther* 7 (2008) 2004-2006.

**Phuoc T. Tran Publications (Continued)**

4. Wendy Hara, **Phuoc Tran**, Gordon Li, Zheng Su, Putipun Puataweepong, John Adler, Steven Chang, Scott Soltys and Iris C. Gibbs. Cyberknife for brain metastases of malignant melanoma and renal cell carcinoma. *Neurosurgery* 64 (2009) A26-32.
5. Melissa Horoschak, **Phuoc T. Tran**, Pavan Bachireddy, Robert B. West, David Mohler, Christopher Beaulieu, Daniel S. Kapp, and Sarah S. Donaldson. External beam radiation therapy enhances local control in pigmented villonodular synovitis. *Int J Radiat Oncol Biol Phys.* 75 (2009) 183-187.
6. Christopher H. Chapman, John Shen, Edith J. Filion, **Phuoc T. Tran**, Wendy Hara, Alfredo Asuncion, Daniel Marko, Heather Wakelee, Gerald J. Berry, Kevin W. Dimmick, Billy W. Loo, Jr., Jon Green. Marked tumor response and fatal hemoptysis during radiation for lung cancer in an HIV-positive patient taking nelfinavir. *J Thorac Oncol* 4 (2009) 1587-1589.
7. Hu Zhou, Manuel Rodriguez, Fred van den Haak, Geoffrey Nelson, Rahil Jogani, Jiali Xu, Xinzhi Zhu, Yongjiang Xian, **Phuoc T. Tran**, Dean W. Felsher, Paul J. Keall, Edward E. Graves. Development of a MicroCT-Based Image-Guided Conformal Radiotherapy System for Small Animals. *Int J Radiat Oncol Biol* 78 (2010) 297-305.
8. Edward E. Graves, Marta Vilalta, Ivana K. Cecic, Janine T. Erler, **Phuoc T. Tran**, Dean Felsher, Leanne Sayles, Alejandro Sweet-Cordero Quynh-Thu Le and Amato J. Giaccia. Hypoxia in models of lung cancer: implications for targeted therapeutics. *Clin Cancer Res* 16 (2010) 4843-52.
9. Pavan Bachireddy, Diane Tseng, Melissa Horoschak, Daniel T. Chang, Albert C. Koong, Daniel S. Kapp, **Phuoc T. Tran**. Orthovoltage intraoperative radiation therapy for pancreatic adenocarcinoma. *Radiation Oncology* 5 (2010) 105.
10. Carsten H. Nielsen, Richard H Kimura, Nadia Withofs, **Phuoc T. Tran**, Zheng Miao, Jennifer Cochran, Zhen Cheng, Dean Felsher, Andreas Kjær, Juergen K. Willmann, Sanjiv S. Gambhir. PET Imaging of Tumor Neovascularization in a Transgenic Mouse Model with a Novel <sup>64</sup>Cu-DOTA-Knottin Peptide. *Cancer Res* 70 (2010) 9022-30.
11. Alfred P. See\*, Jing Zeng, **Phuoc T. Tran**, and Michael Lim. Acute toxicity of second generation HIV protease-inhibitors in combination with radiotherapy: a retrospective case series. *Radiation Oncology* 6 (2011) 25.

**Jieru Wang, MD, PhD****Mentor:** Robert J. Mason, MD**Institution:** National Jewish Medical and Research Center, Denver**Project Title:** Influenza A virus induces innate immune response in differentiated adult human alveolar type II cells and macrophages**PBF Project Summary, May 2011:**

Influenza is a common public health problem. It causes more than 35,000 deaths, 200,000 hospitalizations, and more than \$37.5 billion in economic loss ([www.cdc.gov](http://www.cdc.gov)). Recently, 2009 H1N1 pandemic virus led to over 18,000 verified death according to WHO. The gas exchange cells deep within the human lung are the primary targets for influenza virus. Seasonal strains of the flu, as well as avian flu, target these cells. We examined what effect the influenza virus has upon two specific cell types, alveolar type II cells and macrophages from the same donor. Our work demonstrated that the influenza virus increases inflammation of type II cells and macrophages. However, other repair molecules are also produced, assisting with the eventual healing of the lung tissue. We are

hopeful that our work will lead to the development of novel treatment targets.

***Publications during PBF Fellowship:***

1. **Wang J**, Oberley-Deegan R, Wang S, Nikrad M, Funk CJ, Hartshorn KL, Mason RJ. Differentiated human alveolar type II cells secrete anti-viral IL-29 (IFN-lambda 1) in response to influenza A infection. *J Immunol.* 2009 Feb 1;182 (3):1296-304.
2. **Wang J**, Nikrad M, Phang T, Gao B, Alford T, Ito Y, Edeen K, Travanty E, Kosmider B, Hartshorn K, Mason RJ. Innate immune response to influenza a virus in differentiated human alveolar type II cells. *Am J Respir Cell Mol Biol.* 2011, Jan 14 (Epub ahead of print).
3. MacLeod MKL, McKee AS, David A, **Wang J**, Mason R, Kappler JW, Marrack P. 2010. Aluminum and Monophosphoryl adjuvanted vaccine generates cross-reactive CD8 T cells that protect mice from viral challenge. *Proc Natl Acad Sci U S A.* 2011 May 10;108(19):7914-9. Epub 2011 Apr 25.
4. Yu WCL, Chan RWY, **Wang J**, Nicholls JM, Peiris JAM, Mason RJ, Chan MCW. Viral replication and innate host response in primary alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses. *J Virol.* 2011 May 4. [Epub ahead of print]

## George R. Washko, Jr., MD

**Mentor:** John J. Reilly, Jr., MD, PhD (1988 PBF Fellow)

**Institution:** Brigham and Women's Hospital

**Project Title:** Computed tomographic imaging in COPD



### PBF Project Summary, May 2011:

I am a clinical researcher in smoking related lung disease focused on imaging and image analysis. I have been awarded additional research support both through subcontracts and a R01 from the National Heart Lung and Blood Institute. This funding has allowed me to build my group to include one full time research technician, two computer scientists, and most recently two pulmonary fellows. We have developed innovative software tools that can be used to examine the impact and associations of multiple disease processes on the lung.

Clinical research involves the recruitment of patients with certain characteristics such as a disease diagnosis, exposure to a toxin such as tobacco smoke and a therapeutic intervention with the subsequent assessment of response to that action. A major limitation is that most lung diseases are very heterogeneous in their manifestations. As an example, only 30 to 40 percent of smokers develop chronic obstructive pulmonary disease. Of this afflicted subset, many have normal productive lives while some have fulminant disease progression and suffer premature disability and death. My goal is to use the image analysis tools we have developed to further understand the risk factors for developing COPD and other lung diseases and to further improve the care of patients with lung disease by helping to define which patients would best respond to selected therapies (with minimal adverse side effects).

Our recent focus has moved into examining the vasculature in the lungs. We believe that this is a very dynamic structure that is sensitive to the presence of multiple disease states. Using our software tools we have begun to understand how the lung vasculature remodels in smoking related lung disease and have entered into discussions with industry to determine if such information may lead to new and improved treatments. We are also expanding our examination of the lung vasculature to include such diseases as sickle cell anemia, pulmonary embolism (blood clots that have traveled to the lung) and liver disease which interestingly has been previously demonstrated to affect the lung. We hope that we will continue to broaden the number of diseases that we examine and how they affect the lung. Lung imaging will not be the sole solution to these complex problems but I believe that the tools that my group and other groups have developed will continue to provide insight into lung disease and help to reveal opportunities for new treatment.

### Publications during PBF Fellowship:

1. Kim WJ, Silverman EK, Hoffman E, Criner GC, Mosenifar Z, Sciruba FC, Make BJ, Carey V, Diaz A, Reilly JJ, Martinez FJ, and Washko GR for the NETT Research Group. Computed Tomographic Metrics of Airway Disease and Emphysema in Severe COPD. *Chest* 2009; 136(2):396-404.
2. Washko GR, Dransfield MT, San Jose Estepar R, Diaz A, Matsuoka S, Yamashiro T, Hatabu H, Silverman EK, Bailey WC, and Reilly JJ. Airway wall attenuation: A biomarker of airway disease in subjects with COPD. *J Appl Physio* 2009; 107(1):185-91.
3. Dransfield MT, Moon NH, Han, MK, Harnden S, Criner, GJ, Martinez, FJ, Scanlon PD, Woodruff PG, Washko GR, Connett JE, Anthonisen NR, and Bailey WC for the COPD Clinical Research Network. Superior Immune Response to Protein-conjugate vs. Free Pneumococcal Polysaccharide Vaccine in COPD. *Am J Respir Crit Care Med.* 2009;180(6):499-505.
4. Ross JC, San Jose Estepar R, Diaz A, Westin CF, Kikinis R, Silverman EK, Washko GR. Lung Extraction, Lobe Segmentation and Hierarchical Region Assessment for Quantitative Analysis on High Resolution Computed Tomographic Images. *Med Image Comput Comput Assist Interv Int Conf Med Image Comput Comput Assist Interv.* 2009;12(Pt 2):690-98.

**George Washko Publications (Continued)**

5. Matsuoka S, Yamashiro T, Washko GR, Kurihara Y, Nakajima Y, Hatabu H. Quantitative CT Assessment of Chronic Obstructive Pulmonary Disease Phenotypes. *Radiographics*. 2010;30(1):55-66.
6. GR Washko, DA Lynch, S Matsuoka, J Ross, S Umeoka, A Diaz, FC Scirba, GM Hunninghake, EK Silverman, IO Rosas, and H Hatabu. Identification of Early Interstitial Lung Disease in Smokers from the COPD Gene Study. *Acad Radiol* 2010;17(1):48-53.
7. Matsuoka S, Washko GR, Dransfield MT, Yamashiro T, San Jose Estepar R, Diaz A, Silverman EK, Patz S, Hatabu H. Quantitative CT Measurement of Cross-sectional Area of Small Pulmonary Vessel in COPD: Correlations with Emphysema and Airflow Limitation. *Acad Radiol* 2010;17(1):93-9.
8. Yamashiro T, Matsuoka S, San Jose Estepar R, Diaz A, Newell J, Sandhaus RA, Mergo P, Brantly ML, Murayama S, Reilly JJ, Hatabu H, Silverman EK, Washko, GR. Quantitative airway assessment on computed tomography in patients with  $\alpha$ 1-antitrypsin deficiency. *COPD* 2009;6(6):468-77.
9. Matsuoka S, Washko GR, Yamashiro T, San Jose Estepar R, Diaz A, Silverman EK, Hoffman EA, Fessler HE, Criner GJ, Marchetti N, Scharf SM, Martinez FJ, Reilly JJ, Hatabu H. Pulmonary hypertension and CT measurement of small pulmonary vessels in severe emphysema. *Am J Respir Crit Care Med*. 2010;181(3):218-225.
10. Yamashiro T, Matsuoka S, Bartholmai B, San Jose Estepar R, Ross JC, Diaz A, Murayama S, Silverman EK, Hatabu H, Washko GR. Collapsibility of lung volume by paired inspiratory and expiratory CT scans: correlations with lung function and mean lung density. *Acad Radiol* 2010;17(4):489-495.
11. Washko GR. Diagnostic imaging in COPD. *Seminars in Resp Med* 2010;31(3):276-85.
12. Washko GR, Martinez FJ, Hoffman EA, Loring SL, San Jose Estepar R, Diaz A, Scirba FC, Silverman EK, Han M, DeCamp M, Reilly JJ, for the NETT Research Group. Physiologic and computed tomographic predictors of outcome from lung volume reduction surgery. *Am J Respir Crit Care Med*. 2010;181(5):494-500.
13. Wan ES, Pober BR, Washko GR, Raby BA, Silverman EK. Pulmonary function and emphysema in Williams-Bueren Syndrome. *Am J Med Genet. A*. 2010;152A(3):653-6.
14. Yamashiro T, Matsuoka S, San Jose Estepar R, Dransfield MT, Reilly JJ, Hatabu H, Washko GR. Quantitative assessment of bronchial wall attenuation on thin-section CT: An indicator of airflow limitation in chronic obstructive pulmonary disease. *Am J. Roent* 2010;195(2):363-9.
15. Nishino M, Washko GR, Hatabu H. Volumetric expiratory HRCT of the lung: clinical applications. *Radiol Clin North Am*. 2010;48(1):177-183.
16. Diaz A, Bartholmai B, San Jose Estepar R, Ross J, Matsuoka S, Yamashiro T, Hatabu H, Reilly JJ, Silverman EK, and Washko GR. Relationship of Emphysema and Airway Disease Assessed by CT to Exercise Capacity in Subjects with COPD. *Resp Med* 2010;104(8):1145-51.
17. Cho MH, Washko GR, Hoffman TJ, Criner GJ, Hoffman EA, Martinez, FJ, Laird N, Reilly JJ, and Silverman EK Cluster Analysis in Severe Emphysema Subjects Using Phenotype and Genotype Data: An Exploratory Investigation. *Respiratory Research* 2010;11(1):30.
18. Chandra D, Lipson, DA, Hoffman EA, Hansen-Flaschen J, Scirba FC, DeCamp MM, Reilly JJ, Washko GR for the NETT Research Group. Perfusion scintigraphy and patient selection for lung volume reduction surgery. *Am J Respir Crit Care Med*. 2010;182(7):937-46.
19. Han MK, Agusti A, Calverley PM, Celli BR, Criner G, Curtis JL, Fabbri LM, Goldin JG, Jones PW, MacNee W, Make BJ, Rabe KF, Rennard SI, Scirba FC, Silverman EK, Vestbo J, Washko GR, Wouters EFM, and Martinez FJ. COPD Phenotypes: The future of COPD. *Am J Respir Crit Care Med*. 2010;182(5):598-604.
20. Diaz A, Valim C, Yamashiro T, San Jose Estepar R, Ross J, Matsuoka S, Bartholmai B, Hatabu H, Silverman EK, and Washko GR. Airway count and emphysema assessed by chest CT predicts clinical outcome in smokers. *Chest*. 2010;138(4):880-7.
21. Kim WJ, Hoffman EA, Reilly JJ, Hersh C, DeMeo DD, Washko GR, Silverman EK. Association of COPD candidate genes with CT emphysema and airway phenotypes in severe COPD. *Eur Resp J*. 2010. In Press.
22. Sorheim, IC, DeMeo DL, Washko GR, Litonjua A, Sparrow D, Bowler R, Bakke PS, Pillai SG, Coxson HO, Lomas DA, Silverman EK, and Hersh CP. Polymorphisms in the superoxide dismutase-3 gene are associated with emphysema in COPD. *COPD*. 2010 In press.
23. Kong X, Cho MH, Anderson W, Coxson H, Muller N, Washko G, Hoffman E, Bakke P, Gulsvik A, Lomas DA, Silverman EK, Pillai SG. Genome-wide association study identifies BICD1 as a potential susceptibility gene for emphysema. *Am J Resp Crit Care Med*. 2011;183(1):43-49.

**George Washko Publications (Continued)**

24. Matsuoka S, Yamashiro T, Diaz A, San Jose Estepar R, Ross JC, Silverman EK, Kobayashi Y, Dransfield MT, Bartholmai B, Hatabu H, and Washko GR. Relationship between small pulmonary vascular alteration and aortic atherosclerosis in chronic obstructive pulmonary disease: Quantitative CT analysis. *Acad Radiol*. 2010;18(1);40-6.
25. Dransfield MT, Huang F, Nath H, Singh SP, Bailey WC, and Washko GR. CT emphysema predicts thoracic aortic calcification in smokers with and without COPD. *COPD*. 2010;7(6);404-10.
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## Timothy Eoin West, PhD

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**Institution:** University of Washington School of Medicine

**Project Title:** Pulmonary host defense in melioidosis



### PBF Project Summary, May 2011:

Melioidosis is an emerging bacterial infection that typically causes pneumonia and sepsis. Pneumonia and sepsis count as major contributors to global mortality but are most common in the poorest parts of the world.<sup>1</sup> Endemic in southeast Asia, 40% of melioidosis patients in northeast Thailand die. The causative bacterium, *Burkholderia pseudomallei*, is resistant to many antibiotics and requires prolonged courses of therapy. Thus, novel therapies for melioidosis are sorely needed. The objective of our research, funded by the Francis Family Foundation, has been to expand our understanding of the role of innate immune receptors in melioidosis. We hope that these findings can also be extrapolated to other causes of pneumonia and sepsis. Innate immune receptors are activated by invading bacteria and trigger an immune

response in the host. Our work incorporates cellular assays in the laboratory, mouse models of lung infection, and human genetic studies of Thais with melioidosis. We have used a closely related but less virulent bacterium, *B. thailandensis*, for initial studies, because it is easier to manipulate in the lab than *B. pseudomallei*.<sup>2</sup> We have identified important roles for several innate immune receptors using these methods.<sup>3,4</sup> Notably, our findings emphasize the importance of studying human populations directly, as we have observed that the innate immune receptor TLR4 that others have not found relevant in the mouse model of disease are in fact implicated in human genetic studies. The most salient discovery during this fellowship is that genetic variation in TLR5 is strongly associated with survival from melioidosis. TLR5 is activated by *B. pseudomallei* flagellin and regulates survival in mice. The genetic variant in TLR5 results in a nonfunctional receptor. Thus, this would suggest that TLR5 activation is deleterious in human melioidosis. We are working to confirm this finding and elucidate the mechanism. Based on the initial data, we believe that targeted inhibition of TLR5 carries the potential to improve outcomes from melioidosis. Conceivably this approach may also be beneficial in other related lung infections such as *Pseudomonas aeruginosa* or in systemic infections such as salmonellosis. We are optimistic that continued study of innate immunity in bacterial lung infections will yield additional insights into the mechanisms of disease that can be exploited for preventative or therapeutic purposes. Such scientific inquiry, generously supported by the Francis Family Foundation, may ultimately reduce the burden of these infections on the world's most vulnerable populations.

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