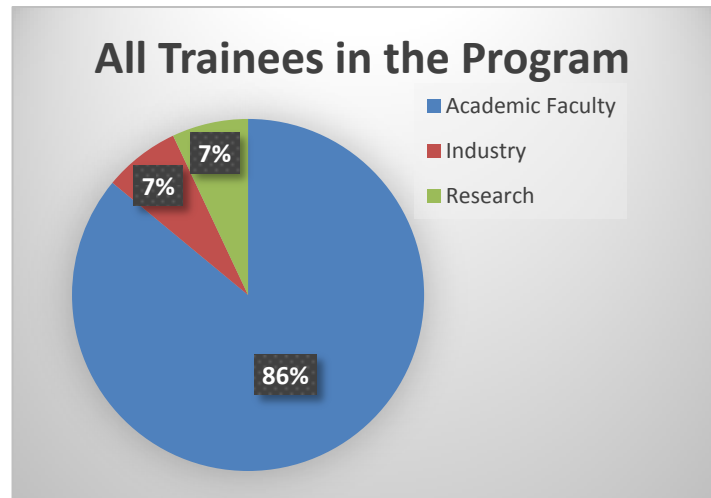# PROGRESS REPORT OF TRAINEES

The UAB Training Program in Lung Biology and Translational Medicine, initiated in September 2010 and successfully renewed for its second cycle in 2014 (09/01/2015 – 08/31/2020), has been extraordinarily successful. <u>Currently in its 10th year, this T32 Training Program has graduated **sixteen** trainees, **fifteen (93%)** of whom are in research-intensive careers – **thirteen** in academic faculty positions, **one** in industry, and **one** at a government agency (FDA)</u>. Our trainees have published over 75 publications related to research conducted during the T32, including high-profile manuscripts in *New England Journal, Cell, Immunity, Nature Medicine, and the Journal of Clinical Investigation*

All Trainees in the Program

- Academic Faculty
- Industry
- Research

7%
7%
86%

and ~80% of these former trainees have obtained independent funding, including 5 former trainees obtaining NIH K-series or R-series awards. Specific information for each former and current trainee is delineated below.

PAST TRAINEES:

Name: WELLS, J. Michael, M.D.   **Appointment Date:** 10/1/2010 – 6/30/2012
Mentor: J. Edwin Blalock, Ph.D.; Mark T. Dransfield, M.D. (Co-Mentor)
Project Title: The Role of Leukotriene A4 Hydrolase in the Pathogenesis of COPD

Primary Research Project: Proline-glycine-proline (PGP) acts as a potent neutrophil chemokine and marker for COPD and substrate for the tri-aminopeptidase (TAP) site of LTA4H, a bifunctional enzyme produced by neutrophils. Normally, LTA4H degrades PGP and leads to resolution of inflammation but cigarette smoke selectively inhibits TAP activity, preventing PGP breakdown and promoting neutrophil accumulation *in vitro* and in animal models. Additionally, the epoxide hydrolase activity of LTA4H promotes production of the pro-inflammatory leukotriene B4, further compounding the effect of cigarette smoke on the lung. We hypothesized that neutrophils, leukotriene A4 hydrolase, and leukotriene B4 would be elevated but the tri-aminopeptidase activity would be diminished in serum and induced sputum from smokers and patients with COPD compared to non-smokers.

The primary aim of this project was to study cigarette smoke mediated inhibition of the TAP activity of leukotriene A4 hydrolase in non-smokers, healthy smokers, COPD former smokers, and COPD current smokers and its role in neutrophilic inflammation and COPD pathogenesis. Clinical samples included sera and induced sputum, collected from patients enrolled through the UAB Lung Health Center. Sputum samples will undergo analysis for neutrophil burden based on myeloperoxidase activity (MPO), leukotriene A4 hydrolase amount by ELISA and a combination immunoprecipitation-Western blot (IP-WB) analysis, TAP activity based on PGP degradation using state-of-the-art mass spectroscopy techniques, and leukotriene B4 concentrations using mass spectroscopy.

A secondary aim of this project was to further characterize the effects of cigarette smoke on leukotriene A4 hydrolase using *in vitro* models and test the reversibility of these modifications. We examined the effects of individual components of cigarette smoke on the aminopeptidase activity using PGP degradation as determined by mass spectroscopy. Also, samples of leukotriene A4 hydrolase were subjected to varying concentrations of cigarette smoke extract and site-specific modifications determined using mass spectroscopy. The mucolytics, carbocysteine and NAC, which have reducing capabilities, were tested to prevent or reverse these modifications and potentially provide a new venue for therapy in the treatment of smoking related airways disease.

Publications (during and as a result of T32-initiated research and training):
1. **Wells JM**, Washko GR, Han MK, Abbas N, Nath H, Mamary AJ, Regan E, Bailey WC, Martinez FJ,

Westfall E, Beaty TH, Curran-Everett D, Curtis JL, Hokanson JE, Lynch DA, Make BJ, Crapo JD, Silverman EK, Bowler RP, Dransfield MT; COPDGene Investigators; ECLIPSE Study Investigators. Pulmonary arterial enlargement and acute exacerbations of COPD. *N Engl J Med.* 2012. PMID: 22938715; PubMed Central PMCID: PMC3690810.

2. **Wells JM**, Dransfield MT. Pathophysiology and clinical implications of pulmonary arterial enlargement in COPD. *Int J Chron Obstruct Pulmon Dis.* 2013. PMID: 24235822; PubMed Central PMCID: PMC3826513.

3. O'Reilly PJ, Jackson PL, **Wells JM**, Dransfield MT, Scanlon PD, Blalock JE. Sputum PGP is reduced by azithromycin treatment in patients with COPD and correlates with exacerbations. *BMJ Open* 2013. PMID: 24366582; PubMed Central PMCID: PMC3884851.

4. Iyer AS, **Wells JM**, Vishin S, Bhatt SP, Wille KM, Dransfield MT. CT scan-measured pulmonary artery to aorta ratio and echocardiography for detecting pulmonary hypertension in severe COPD. *Chest* 2014. PMID: 24114440; PubMed Central PMCID: PMC3971971.

5. Bhatt SP, Cole AG, **Wells JM**, Nath H, Watts JR, Cockcroft JR, Dransfield MT. Determinants of arterial stiffness in COPD. *BMC Pulm Medicine* 2014. PMID: 24387157; PubMed Central PMCID: PMC3890490.

6. **Wells JM**, Jackson PL, Viera L, Bhatt SP, Gautney J, Handley G, King RW, Xu X, Gaggar A, Bailey WC, Dransfield MT, Blalock JE. A randomized, placebo-controlled trial of roflumilast: Effect of proline-glycine-proline and neutrophilic inflammation in chronic obstructive pulmonary disease. *AJRCCM* 2015. PMID: 26151090; PMCID: PMC4642210.

7. **Wells JM**, Iyer AS, Rahaghi FN, Bhatt SP, Gupta H, Denney TS, Lloyd SG, Dell'Italia LJ, Nath H, Estepar RS, Washko GR, Dransfield MT. Pulmonary artery enlargement is associated with right ventricular dysfunction and loss of blood volume in small pulmonary vessels in chronic obstructive pulmonary disease. *Circ Cardiovasc Imaging* 2015. PMID:25855668; PMCID: PMC4392846

Professional Development: Dr. Wells was recruited into the T32 Training Program during his second year of Pulmonary and Critical Care Medicine fellowship training based on his strong desire and aptitude for academic research career. As a result of work during his T32 research training, Dr. Wells published a first-author paper in the *New England Journal of Medicine* in 2012 in which he showed that pulmonary artery enlargement (pulmonary artery : aorta ratio of >1), as detected by computed tomography (CT), was associated with severe exacerbations of COPD. He is actively studying the role of PGP and leukotriene A4 hydrolase in pulmonary vascular remodeling utilizing animal models of emphysema and in COPD patents. During the second year on this T32 Training Grant, Dr. Wells successfully competed for the highly prestigious Walter B. Frommeyer Award from the Department of Medicine to investigate the effect of roflumilast on neutrophilic inflammation, focusing on derangements to the PGP-LTA4H pathway. Based on his outstanding productivity and securing salary support with the Frommeyer Award, we were able to transition him off the T32 Training Grant prior to the completion of his second year on the T32. Dr. Wells was appointed as an Assistant Professor of Medicine in the PACCM Division, at UAB on July 1, 2012 and, most recently, promoted to Associate Professor of Medicine in September 2018. Since completion of his T32 training period, Dr. Wells obtained a K08 NIH Clinical Scientist Research Career Development Award in 2014, focusing the role of PGP peptides on the development of COPD-related pulmonary hypertension. This work has led to multiple manuscripts including recent papers in *JCI-Insight*, *Lancet Respiratory Medicine*, *American Journal of Respiratory and Critical Care*, and *Cell*. Dr. Wells has just received intent to fund his first R01 proposal, and now serves as training faculty on this T32 grant. Additionally, he serves as a Co-Investigator on a NIH-UH3 award to a first-in-class disease modifying therapy to treat alpha1-antitrypsin deficiency.

Name: KLIMENTIDIS, Yann C., Ph.D.   **Appointment Date:** 1/1/2011 – 12/31/2011
Mentor: David B. Allison, Ph.D; Mark T. Dransfield, M.D., and Victor J. Thannickal, M.D. (Co-Mentors)
Project Title: Heritability of Lung Function Based on Genome-Wide Markers

Primary Research Project: Prediction of phenotypic traits from genetic information remains a major challenge despite all the recent advances in genotyping technology. However, with the recent development of statistical methods to predict phenotypic traits from genotypic data, it has become possible to use many thousands of

SNP makers at once to build a predictive model in a training dataset, and test the predictive ability of this model in a testing dataset. In this T32 Training Grant project, Dr. Klimentidis proposed to use thousands of genetic markers at once to build predictive models for the heritability of pulmonary function testing. Dr. Klimentidis tested these models using data from the Framingham Heart Study dataset, which consists of approximately 7,500 subjects. He used novel methods to estimate the heritability of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and the ratio of these two measures (FEV1/FVC) among subjects in the Framingham Heart Study dataset. Heritability estimates based on pedigree-based relationships and those based on genome-wide SNPs. He found that estimates of heritability using SNP data are nearly identical to estimates based on pedigree information, and range from 0.50 for FEV1 to 0.66 for FEV1/FVC. These findings, published in *Frontiers in Genetics*, suggest a larger genetic basis for FEV1/FVC and show that it is possible to capture most of the genetic variability in these traits using information from SNP genotypes. These models will pave the way towards potentially using an individual's genetic information to predict pulmonary-related outcomes.

Publications (during and as a result of T32-initiated research and training):
1. **Klimentidis YC**, Dulin-Keita A, Casazza K, Willig AL, Allison DB, Fernandez JR. Genetic admixture, social-behavioral factors and body composition are associated with blood pressure differently by racial-ethnic group among children. *J Hum Hypertension* 2012. PMID: 21248781; PubMed Central PMCID: PMC3172395.
2. de los Campos G, **Klimentidis YC**, Vazquez AI, Allison DB. Prediction of expected years of life using whole-genome markers. *PLoS One* 2012. PMID: 22848416; PubMed Central PMCID: PMC3405107.
3. **Klimentidis YC**, Vazquez AI, de Los Campos G, Allison DB, Dransfield MT, Thannickal VJ. Heritability of pulmonary function estimated from pedigree and whole-genome markers. *Front Genetics* 2013. PMID: 24058366; PubMed Central PMCID: PMC3766834.
4. de Los Campos G, Vazquez AI, Fernando R, **Klimentidis YC**, Sorensen D. Prediction of complex human traits using the genomic best linear unbiased predictor. *PLoS Genetics* 2013.PMID: 23874214; PubMed Central PMCID: PMC3708840.
5. **Klimentidis YC**, Lemas DJ, Wiener HH, O'Brien DM, Havel PJ, Stanhope KL, Hopkins SE, Tiwari HK, Boyer BB. CDKAL1 and HHEX are associated with type 2 diabetes-related traits among Yup'ik people. *J Diabetes* 2014. PMID: 24112421; PubMed Central PMCID: PMC3964139.
6. **Klimentidis YC**, Wineinger NE, Vazquez AI, de Los Campos G. Multiple metabolic genetic risk scores and type II diabetes risk in three racial/ethnic groups. *J Clin Endocrinol Metab* 2014. PMID: 24905067; PubMed Central PMCID: PMC4154088.
7. **Klimentidis YC**, Zhou J, Wineinger NE. Identification of alleic heterogenity at type-2 diabetes loci and impact of prediction. *PLOS One* 2014. PMID: 25393876; PubMed Central PMCID: PMC4231111

Professional Development: Dr. Klimentidis was appointed on this T32 Training Grant for a period of one year (1/1/11 to 12/31/11); he worked with Dr. Allison in the Division of Statistical Genetics, Department of Biostatistics (SOPH) as a post-doctoral fellow for 2 years prior to joining our program. Dr. Klimentidis was highly productive in his research which was focused on applying novel statistical methods to increase our ability to use genetic information for predicting and understanding complex traits, such as pulmonary function. His success on this T32 program and his overall outstanding research productivity resulted in the rapid transition to a faculty position as Instructor, Office of Energetics, School of Public Health at UAB, beginning 1/1/12. Dr. Klimentidis has been awarded a NIH K01 career development award to develop and test prediction models to assess genetic risk in complex diseases. He is currently a tenure-track Assistant Professor in the Division of Epidemiology and Biostatistics at the University of Arizona. He is currently funded on R01 HL136528 (4/1/18 – 1/31/21) to study genetics at the interface of lipid and glycemic traits.

Name: MYERS, Riley C., Ph.D.   **Appointment Date:** 10/1/2011 – 6/30/2013
Mentor: Chad Steele, Ph.D.; Troy D. Randall, Ph.D. (Co-Mentor)
Project Title: Adaptive Immune Responses against the Lung Fungal Pathogen *Pneumocystis Murina*

Primary Research Project: Although it is clear that the loss of CD4+ T cells is a predisposing factor for the development of Pneumocystis pneumonia, the protective mechanisms mediated by CD4+ T cells are not well understood. Augmented production of Th1-type cytokines in the lungs of IL-10$^{-/-}$ mice correlated with enhanced P. murina clearance, whereas neutralization of IL-17 in normal mice compromised fungal clearance, suggesting that Th1 and Th17 cells play an important role during infection. STAT4 is critical for optimal Th1 and Th17 development, therefore, we investigated its role in P. murina host defense. Our data shows that STAT4 was required for Th1 and, unexpectedly, Th2 responses in the lungs of C57BL/6 (BL/6) and Balb/c mice 14 days after infection; however, only Balb/c STAT4$^{-/-}$ mice were susceptible to P. murina infection. Th2 responses in the lungs of BL/6 STAT4$^{-/-}$ mice, but not Balb/c STAT4-/- mice, were intact 28 days after infection and correlated with elevated M2 macrophage polarization. Additionally, anti-Pseudomonas murina class-switched antibodies were increased in BL/6 STAT4$^{-/-}$ mice, but not Balb/c STAT4$^{-/-}$ mice, as a result of hyper-Th2 responses in the draining lymph nodes. Supporting our experimental observations, we found that lower IL- 4, IL-5 and IL-13 levels in plasma from HIV+ patients correlated with Pneumocystis colonization. Collectively, these data suggests that robust local and systemic Th2-mediated responses are critical for immunity to Pneumocystis.

Publications (during and as a result of T32-initiated research and training):
1. **Myers RC**, King RG, Carter RH, Justement LB. Lymphotoxin α1β2 expression on B cells is required for follicular dendritic cell activation during the germinal center response. *Eur J Immunology* 2013 PMID: 23112125; PubMed Central PMCID: PMC3753018.
2. **Myers RC**, Dunaway CW, Nelson MP, Trevor JL, Morris A, Steele C. STAT4-dependent and – independent Th2 responses correlate with protective immunity against lung infection with Pneumocysti murina. *J Immunology* 2013. PMID: 23650614; PubMed Central PMCID: PMC3679253
3. Heesters BA, **Myers RC**, Carroll MC. Follicular dendritic cells: dynamic antigen libraries. *Nat Rev Immunology* 2014. PMID:24948364.

Professional Development: After receiving his Ph.D. studying receptor-mediated activation of follicular dendritic cells (FDCs), Dr. Myers sought post-doctoral training in Dr. Steele's lab in the area of pathogen-associated mucosal immunology. Following his Pulmonary T32 training, Dr. Myers took a position as <u>Senior Research Scholar at Children's Hospital of Boston/Harvard University where he has combined his doctoral and post-doctoral research programs</u> into currently understanding how pathogen-associated molecular pathogen signaling in FDCs upregulates factors that promote B cell survival and somatic hypermutation. Dr. Myers currently <u>works for the U.S. Food and Drug Administration (FDA) as a staff microbiologist</u>.

Name: BRATCHER, Preston E., Ph.D.   **Appointment Date:** 11/1/2011 – 10/31/2014
Mentor: Amit Gaggar, M.D., Ph.D.; Steven Rowe, M.D. (Co-Mentor)
Project Title: Alterations in the Pulmonary Innate Immunity of Patients with Cystic Fibrosis

Primary Research Project: Progressive obstructive lung disease is the predominant source of morbidity and mortality observed in cystic fibrosis (CF) lung disease. Bacterial colonization and subsequent inflammation plays a central role in the ongoing damage seen in the CF lung. The overarching goal of this project is to examine the extent/impact of matrix metalloprotease-9 (MMP-9)-mediated cleavage of surfactant protein-D (SP-D) in the CF lung. Our *in vitro* experiments have shown that MMP-9 is able to fragment SP-D in a time- and dose-dependent manner, and that these fragments lose their ability to agglutinate bacteria and increase phagocytosis by macrophages. In addition, we have characterized the relative contribution of MMP-9 to the degradation of SP-D in supernatants from stimulated primary human neutrophils, exhibiting *ex vivo* relevance for our *in vitro* results (manuscript in preparation for submission for publication). Further studies are in progress to examine potential alterations in the pulmonary inflammatory response caused by cleavage of SP-D by MMP-9 *in vivo*. These potential alterations include differences in macrophage phenotype, release of inflammatory cytokines by inflammatory cells, and influx of neutrophils and monocytes into the lung. In addition, experiments are currently being performed to analyze SP-D in *ex vivo* CF patient sputum samples. Preliminary results have shown that incubation of CF sputum at 37°C results in time-dependent generation of

SP-D fragments, and the addition of a specific inhibitor of MMP-9 decreases the rate of degradation. Additional experiments are being performed to determine the other proteases responsible for this cleavage and the relative contributions of these proteases to SP-D degradation. The results from these experiments will determine if targeting MMP-9 in CF may augment the maintenance of SP-D for improved host defense.

Publications (during and as a result of T32-initiated research and training):
1. **Bratcher PE**, Weathington NM, Nick HJ, Jackson PL, Snelgrove RJ, Gaggar A. MMP-9 cleaves SP-D and abrogates its innate immune functions in vitro. *PLOS One* 2012;PMID: 22860023; PubMed Central PMCID: PMC3408449.
2. **Bratcher PE**, Gaggar A. Characterization and prevention of the adsorption of surfactant protein D to polypropylene. *PLOS One* 2013. PMID: 24039953; PubMed Central PMCID: PMC3770593.
3. **Bratcher PE**, Gaggar A. Factors influencing the measurement of plasma/serum surfactant protein D levels by ELISA. *PLOS One* 2014. PMID: 25365324; PubMed Central PMCID: PMC4218753.
4. **Bratcher PE**, Rowe SM, Reeves G, Roberts T, Szul T, Harris WT, Tirouvanziam R, Gaggar A. Alterations in blood leukocytes of G551D-bearing cystic fibrosis patients undergoing treatment with ivacaftor. J Cyst Fibrosis 2016. PMID: 25769931; PubMed Central PMCID: PMC4567518.
5. Szul T, **Bratcher PE**, Fraser KB, Kong M, Tirouvanziam R, Ingersoll S, Sztul E, Rangarajan S, Blalock JE, Xu X, Gaggar A. Toll-Like Receptor 4 engagement mediates prolyl endopeptidase release from airway epithelia via exosomes. Am J Respir Cell Mol Biology 2016. PMID: 26222144; PubMed Central PMCID: PMC5455678.
6. Xu X, Abdalla T, **Bratcher PE**, Jackson PL, Sabbatini G, Wells JM, Lou XY, Quinn R, Blalock JE, Clancy JP, Gaggar A. Doxycycline improves clinical outcomes during cystic fibrosis exacerbations. Eur Respir Journal 2017. PMID: 28381428.

Professional Development: Since enrolling in this T32 Training Program, Dr. Bratcher has generated exciting new data on the regulation of immunity in CF lung disease, specifically in the regulation of SP-D by proteases. In addition to the published manuscripts, Dr. Bratcher has presented his work at national/international meetings of the American Thoracic Society and at the American Academy of Allergy, Asthma & Immunology; recently, he was also invited to present his research at Baylor College of Medicine, Division of Pulmonary and Critical Care Medicine. Dr. Bratcher became Instructor in Pediatrics at National Jewish Hospital in Denver, CO in 2015, developing an independent laboratory. In 2018, Dr. Bratcher received the prestigious Gilead Scholars Award in Cystic Fibrosis Research and was promoted to Assistant Professor of Pediatrics at National Jewish. He plans to submit an R01 grant application in early 2020.

Name: DESAI, Leena P., Ph.D.   **Appointment Date:** 5/1/2012 – 4/30/2015
Mentor: Victor J. Thannickal M.D.; Yong Zhou, Ph.D. (Co-Mentor, junior)
Project Title: Role of Hic-5 in Myofibroblast Senescence and Fibrogenesis

Primary Research Project: Hydrogen peroxide-inducible clone-5 (Hic-5) is a focal adhesion adaptor protein induced by the pro-fibrotic cytokine, transforming growth factor-β1 (TGF-β1). Hic-5 has also been implicated in fibroblast senescence. However, the precise roles of Hic-5 in fibrogenic activities and senescence of fibroblasts remain unclear. Previous studies in the Thannickal laboratory demonstrated that TGF-β1-induced myofibroblast differentiation and lung fibrosis is mediated by the activation of a reactive oxygen species (ROS)-generating enzyme, NADPH oxidase 4 (Nox4). In this project, Dr. Desai investigated the role of Hic-5 in regulating Nox4, myofibroblast differentiation and senescence. In normal human diploid fibroblasts, TGF-β1 induces Hic-5 expression in parallel with increased levels of Nox4, α-smooth muscle actin (α-SMA) and fibronectin. Hic-5 silencing induced constitutively higher levels of Nox4 and augmented TGF-β1-induced Nox4 in association with markers of myofibroblast differentiation and senescence. This negative regulation of Nox4 by Hic-5 was independent of transcription and translation, and controlled post-translationally by the ubiquitin-proteasomal system (UPS). Although Hic-5 silencing induced markers of autophagy, the lysosomal pathway does not appear to regulate steady-state levels of Nox4 protein. Hic-5 associates with the ubiquitin ligase, Cbl-c, and the ubiquitin-binding protein, heat shock protein 27 (HSP27). The interaction of these proteins is required for the ubiquitination of Nox4 and for maintaining low basal levels of this ROS-generating enzyme.

Our model suggests that TGF-β1-induced Hic-5 functions as a negative feedback mechanism to limit myofibroblast differentiation and senescence by promoting UPS-mediated degradation of Nox4. Immunohistochemistry (IHC) of lung tissues from human subjects with a progressive fibrotic disease, idiopathic pulmonary fibrosis (IPF), demonstrates decreased expression of Hic-5 in fibroblastic foci. Together, these studies indicate that endogenous Hic-5 suppresses senescence and pro-fibrotic activities of myofibroblasts by down-regulating Nox4 protein expression. Additionally, these are the first studies, to our knowledge, to demonstrate post-translational regulation of Nox4. These findings identify Hic-5 and Cbl-c as potential targets for therapeutic intervention in resolving fibrosis and to promote tissue homeostasis in the lung.

Publications (during and as a result of T32-initiated research and training):
1. Zhou Y, Huang X, Hecker L, Kurundkar D, Kurundkar A, Liu H, Jin TH, **Desai L**, Bernard K, Thannickal VJ. Inhibition of mechanosensitive signaling in myofibroblasts ameliorates experimental pulmonary fibrosis. *J Clin Investigation* 2013. PMID: 23434591; PubMed Central PMCID: PMC3582144
2. Sanders YY, Liu H, Zhang X, Hecker L, Bernard K, **Desai L**, Liu G, Thannickal VJ. Histone modifications in senescence-associated resistance to apoptosis by oxidative stress. *Redox Biology* 2013. PMID: 24024133; PubMed Central PMCID: PMC3757696.
3. **Desai LP**, Zhou Y, Estrada AV, Ding Q, Cheng G, Collawn JF, Thannickal VJ. Negative regulation of NADPH oxidase 4 by hydrogen peroxide-inducible clone 5 (Hic-5) protein. *J Biol Chemisty* 2014. PMID: 24831009; PubMed Central PMCID: PMC4140251.
4. Hu M, Che P, Han X, Cai GQ, Liu G, Antony V, Luckhardt T, Siegal GP, Zhou Y, Liu RM, **Desai LP**, O'Reilly PJ, Thannickal VJ, Ding Q. Therapeutic targeting of SRC kinase in myofibroblast differentiation and pulmonary fibrosis. *J Pharmacol Exp Therapeutics* 2014 PMID: 25047515; PubMed Central PMCID: PMC4165028.
5. Liu RM, **Desai LP**. Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biology* 2015, PMID: 26496488; PubMed Central PMCID: PMC4625010.

Professional Development: Dr. Desai has elucidated a novel pathway for the post-translational regulation of the pro-fibrotic mediator, Nox4. She has presented her work at the International Meeting of the American Thoracic Society, selected for a poster discussion session entitled, "Mechanisms that Link Aging with Lung Disease: The Cutting Edge". In addition to publications in the *Journal of Clinical Investigation* and *Redox Biology*, she has published a first-author book chapter related to molecular aspects of aging, and a first-author publication in the *Journal of Biological Chemistry*. After completing her Pulmonary T32 Training, she submitted a K01 grant application to the National Institutes of Aging and remained on the faculty at UAB for a year (through April, 2016), prior to moving to North Carolina; she is in the process of seeking a faculty position in the Department of Biochemistry and Biophysics at the University of North Carolina.

Name: BIRKET, Susan E., Ph.D.   **Appointment Date:** 9/1/2012 – 8/31/2014
Mentor: Steven M. Rowe, M.D., M.S.P.H. (Mentor); Eric J. Sorscher (Co-Mentor)
Project Title: Mechanisms Underlying Mucociliary Clearance in Cystic Fibrosis

Primary Research Project: Cystic fibrosis (CF) is characterized by abnormal mucociliary clearance (MCC), secondary to ion transport defects, leading to pulmonary dysfunction. Conventional models suggest that decreased MCC is due to reduced periciliary layer (PCL) and airway surface layer (ASL) depth. However, controversy surrounds these observations, with some evidence showing normal PCL in novel CF models. Investigation has been limited by lack of imaging technology that can simultaneously measure PCL, ciliary dynamics, and MCC. Current methods are unable to capture these measurements in living tissues under the normal shear stress caused by breathing. Using Micro-Optical Coherence Tomography (μOCT), a high-resolution reflectance imaging modality co-invented by the Rowe laboratory, we are able to simultaneously evaluate PCL and ASL along with ciliary dynamics and MCC in trachea of CF and non-CF animal models under physiologic conditions, including shear stress, to characterize the functional microanatomy underlying CF. Data obtained from μOCT imaging have shown that there is a relationship between PCL and MCT. PCL is increased in adult and piglet trachea subjected to shear stress, compared to controls. MCT is also increased in tissues subjected to shear stress as well. In normal tracheae, PCL depth is linearly related to MCT rate, a

crucial relationship not previously established. In CF tracheae, the relationship between PCL depth and MCT is completely disrupted, with greater PCL depths associated with the slowest transport. Furthermore, CF mucus viscosity is significantly greater than non-CF mucus when monitored in situ, suggesting there is a fundamental difference of mucus in CF trachea compared with controls. We hypothesize that the difference in viscosity is secondary to lack of bicarbonate ion secretion that occurs with the CF defect. These findings could have significant implications on our understanding of CF pathogenesis and therapeutics, in addition to other diseases of mucociliary clearance.

Publications (during and as a result of T32-initiated research and training):

1. Liu L, Chu K, Houser G, Diephuis Y, Wilsterman E, Shastry S, Dierksen G, **Birket SE**, Mazur M, Byan-Parker S, Grizzle W, Sorscher E, Rowe S, Tearney G. Method for quantitative study of airway functional microanatomy using micro-optical coherence tomography. *PLOS One* 2013. PMID: 23372732.
2. Tuggle KL, **Birket SE**, Cui X, Hong J, Warren J, Reid L, Chambers A, Ji D, Gamber K, Chu KK, Tearney G, Tang LP, Fortenberry JA, Du M, Cadillac JM, Bedwell DM, Rowe SM, Sorscher EJ, Fanucchi MV. Characterization of defects in ion transport and tissue development in cystic fibrosis transmembrane conductance regulator (CFTR)-knockout rats. *PLoS One* 2014. PMID: 24608905; PubMed Central PMCID: PMC3946746.
3. Liu L, Shastry S, Byan-Parker S, Houser G, K Chu K, **Birket SE**, Fernandez CM, Gardecki JA, Grizzle WE, Wilsterman EJ, Sorscher EJ, Rowe SM, Tearney GJ. An autoregulatory mechanism governing mucociliary transport is sensitive to mucus load. *Am J Respir Cell Mol Biol* 2014. PMID: 24937762; PubMed Central PMCID: PMC4189485.
4. **Birket SE**, Chu KK, Liu L, Houser GH, Diephuis BJ, Wilsterman EJ, Dierksen G, Mazur M, Shastry S, Li Y, Watson JD, Smith AT, Schuster BS, Hanes J, Grizzle WE, Sorscher EJ, Tearney GJ, Rowe SM. A functional anatomic defect of the cystic fibrosis airway. *Am J Respir Crit Care Med* 2014. PMID: 25029666; PubMed Central PMCID: PMC4214131.
5. Keiser NW, **Birket SE**, Evans IA, Tyler SR, Crooke AK, Sun X, Zhou W, Nellis JR, Stroebele EK, Chu KK, Tearney GJ, Stevens MJ, Harris JK, Rowe SM, Engelhardt JF. Defective innate immunity and hyperinflammation in newborn cystic fibrosis transmembrane conductance regulator-knockout ferret lungs. *Am J Respir Cell Mol Biol* 2015. PMID: 25317669; PubMed Central PMCID: PMC4491130.
6. **Birket SE**, Chu KK, Houser GH, Liu L, Fernandez CM, Solomon GM, Lin V, Shastry S, Mazur M, Sloane PA, Hanes J, Grizzle WE, Sorscher EJ, Tearney GJ, Rowe SM. Combination therapy with cystic fibrosis transmembrane conductance regulator modulators augment the airway functional microanatomy. *Am J Physiol Lung Cell Mol Physiol* 2016. PMID: 26968770; PubMed Central PMCID: PMC4896103.
7. Lewis WR, Malarkey EB, Tritschler D, Bower R, Pasek RC, Porath JD, **Birket SE**, Saunier S, Antignac C, Knowles MR, Leigh MW, Zariwala MA, Challa AK, Kesterson RA, Rowe SM, Drummond IA, Parant JM, Hildebrandt F, Porter ME, Yoder BK, Berbari NF. Mutation of Growth Arrest Specific 8 Reveals a Role in Motile Cilia Function and Human Disease. *PLoS Genet* 2016. PMID: 27472056; PubMed Central PMCID: PMC4966937.
8. Solomon GM, Francis R, Chu KK, **Birket SE**, Gabriel G, Trombley JE, Lemke KL, Klena N, Turner B, Tearney GJ, Lo CW, Rowe SM. Assessment of ciliary phenotype in primary ciliary dyskinesia by micro-optical coherence tomography. *JCI Insight* 2017. PMID: 28289722; PubMed Central PMCID: PMC5333960.
9. Krick S, Baumlin N, Aller SP, Aguiar C, Grabner A, Sailland J, Mendes E, Schmid A, Qi L, David NV, Geraghty P, King G, **Birket SE**, Rowe SM, Faul C, Salathe M. Klotho inhibits IL-8 secretion from cystic fibrosis airway epithelia. *Sci Rep* 2017. PMID: 29085059; PubMed Central PMCID: PMC5662572.

Professional Development: Dr. Birket has identified a mechanism by which ion transport, particularly bicarbonate, through the CFTR alters mucus properties and clearance in animal models of the CF airway. In addition to using the novel µOCT imaging technology to further characterize existing animal models of CF, Dr. Birket has developed a new CFTR knockout rat model, which will be invaluable to studies of mucus production and clearance, as well as CF airway pathophysiology. She has presented her work at the annual North American Cystic Fibrosis Conferences and the American Thoracic Society meetings. She has <u>obtained</u>

Name: SOLOMON, George M., M.D.   **Appointment Date:** 8/1/2013 – 6/30/2014
Mentor: Steven M. Rowe, M.D., M.S.P.H.; David Bedwell, Ph.D. (Co-Mentor)
Project Title: Primary Human Nasal Epithelial Cells as an Outcome Tool for Individualized CFTR Therapies

Primary Research Project: The approval of ivacaftor for the treatment of CF subjects with the G551D mutation indicates the strong potential of CFTR modulators to improve clinical outcomes in CF. However, development of small molecules that target the most common CFTR mutations has been more challenging. Therefore, it is likely that multi-drug combination therapy will be necessary for the effective treatment of subjects homozygous for F508del CFTR. Sensitive biomarkers to detect altered CFTR function in response to therapeutic agents of various medication classes will be needed. Unfortunately, in-vivo assays, including sweat chloride and nasal potential difference, have exhibited challenges in this regard, particularly in detecting efficacy of corrector therapy for F508del CFTR. Furthermore, both biomarkers demonstrate a poor correlation to clinical improvement on an individualized basis, which represents a major impediment to the development and prioritization of CFTR therapeutics. To develop a personalized approach to rational drug design in an era of multi-agent CFTR therapeutics, improved biomarkers of CFTR modulation that overcome these limitations will be needed. This project proposes to address these challenges by using primary human epithelial cell culture of nasal origin (HNE) which maintain an airway phenotype when placed at air-liquid interface; combined with state-of-the art methods of determining CFTR activity and functional microanatomy, including mucociliary transport.

Publications (during and as a result of T32-initiated research and training):
1. Birket SE, Chu KK, Houser GH, Liu L, Fernandez CM, **Solomon GM**, Lin V, Shastry S, Mazur M, Sloane PA, Hanes J, Grizzle WE, Sorscher EJ, Tearney GJ, Rowe SM. Combination therapy with cystic fibrosis transmembrane conductance regulator modulators augment the airway functional microanatomy. *Am J Physiol Lung Cell Mol Physiol* 2016. PMID: 26968770; PubMed Central PMCID: PMC4896103.
2. **Solomon GM**, Hathorne H, Liu B, Raju SV, Reeves G, Acosta EP, Dransfield MT, Rowe SM. Pilot evaluation for ivacaftor in chronic bronchitis. *Lancet Resp Medicine* 2016. PMID:27185048; PubMed Central PMCID: PMC4916910
3. Chu KK, Unglert C, Ford TN, Cui D, Carruth RW, Singh K, Liu L, Birket SE, **Solomon GM**, Rowe SM, Tearney GJ. In vivo imaging of airway cilia and mucus clearance with micro-optical coherence tomography. *Biomed Opt Express* 2017. PMID:27446685; PubMed Central PMCID: PMC4948609
4. Cui D, Chu KK, Yin B, Ford TN, Hyun C, Leung HM, Gardecki JA, **Solomon GM**, Birket SE, Liu L, Rowe
   SM, Tearney GJ. Flexible, high-resolution micro-optical coherence tomography endobronchial probe toward in vivo imaging of cilia. *Opt Letters* 2017. PMID: 28198885; PubMed Central PMCID: PMC5665567.
5. Donaldson SH, **Solomon GM**, Zeitlin PL, Flume PA, Casey A, McCoy K, Zemanick ET, Mandagere A, Troha JM, Shoemaker SA, Chmiel JF, Taylor-Cousar JL. Pharmokinetics and safety of cavosonstat (N91115) in healthy and cystic fibrosis adults homozygous for F508DEL-CFTR. *J Cystic Fibrosis* 2017. PMID: 28209466.
6. **Solomon GM**, Francis R, Chu KK, Birket SE, Gabriel G, Trombley JE, Lemke KL, Klena N, Turner B, Tearney GJ, Lo CW, Rowe SM. Assessment of ciliary phenotype in primary ciliary dyskinesia by micro-optical coherence tomography. *JCI Insight* 2017. PMID: 28289722; PubMed Central PMCID: PMC5333960.

Professional Development: Dr. Solomon was recruited to this T32 Training Program in the fall of 2013, after completing a clinical and research fellowship in Pulmonary and Critical Care Medicine at the University of Colorado/National Jewish Health where he was also a T32 trainee; he was eligible for a total of 2 years on the

current T32 grant at UAB. Through this training, Dr. Solomon has gained experience and expertise in epithelial biology and acquired techniques of developing cell-based personalized therapeutics in human subjects. He has established collaborations to explore novel methods of basal cell maintenance and differentiation, and developed these cell systems during his T32 training. <u>Dr. Solomon is currently an Assistant Professor of Medicine in the Division of Pulmonary, Allergy, and Critical Care Medicine at UAB</u>. Dr. Solomon is establishing a non-CF bronchiectasis clinic that will specialize in PCD and other mucociliary clearance disorders. This clinic will complement his scientific interests, enabling clinical translation of his basic studies with bronchial epithelial cells. <u>He has obtained CF Foundation funding in the past and obtained an NIH-K08 award (HL138153; 1/7/19-12/31/23) to conduct studies of mucociliary transport in primary ciliary dyskinesia</u>.

Name: **RANGARAJAN, Sunad, M.D.**   **Appointment Date:** 7/1/2014 – 6/30/2016
Mentor: Victor J. Thannickal, M.D.; Jaroslaw Zmijewski, Ph.D. (Co-Mentor)
Project Title: Uncoupling Proteins and Mitochondrial Dysfunction in IPF

Primary Research Project: Aging is a major risk factor for idiopathic pulmonary fibrosis (IPF), with a marked increase in incidence and prevalence in aged populations. Despite this strong association, cellular/molecular mechanisms that account for the aging predilection to fibrotic disease are only now beginning to be explored. Mitochondrial dysfunction and altered bioenergetics are common to aging and IPF. Dr. Rangarajan demonstrated that the mitochondrial uncoupling protein-2 (UCP2) is increased in lung fibroblasts (Fbs) of IPF patients, and in normal lung Fbs stimulated with TGF-β1. UCP2 silencing inhibits myofibroblast differentiation and senescence, decreased NADPH oxidase-4 (Nox4) expression, and reprograms metabolism for more efficient oxidative phosphorylation with decreased glycolytic flux. Importantly, UCP2 silencing promotes resolution of fibrosis in aged mice with otherwise persistent fibrosis. Dr. Thannickal's laboratory had previously shown that Nox4 is a key mediator of myofibroblasts differentiation, senescence and resistance to apoptosis in age-related lung fibrosis. In this project, Dr. Rangarajan will test the hypothesis that induction of UCP2 during lung injury-repair in aging contributes to bioenergetic dysfunction, sustained myofibroblast senescence and apoptosis resistance, leading to age-associated persistent fibrosis. In a related project, he showed that the FDA-approved protein tyrosine kinase inhibitor, Nintedanib, a triple receptor tyrosine kinase (VEGF, FGF and PDGF) inhibitor induces increased autophagic flux in IPF fibroblasts. Dr. Rangarajan's data shows that induction of autophagy and the anti-fibrotic effects induced by Nintedanib are independent of classical autophagy pathways involving Becli-1 and ATG6. His studies, published in *Nature Medicine*, show that activation of AMP-activated protein kinase (AMPK), a bioenergetic sensor and regulator of lipid and carbohydrate metabolism, accelerates resolution of lung fibrosis by promoting mitochondrial biogenesis and augmenting autophagy.

Publications (during and as a result of T32-initiated research and training):
1. Sanders YY, Cui Z, Le Saux CJ, Horowitz JC, **Rangarajan S**, Kurundkar A, Antony VB, Thannickal VJ. SMAD-independent down-regulation of caveolin-1 by TGF-β: effects on proliferation and survival of myofibroblasts. *PLOS One* 2015. PMID: 25658089; PubMed Central PMCID: PMC4319960.
2. **Rangarajan S**, Kurundkar A, Kurundkar D, Bernard K, Sanders YY, Ding Q, Antony VB, Zhang J, Zmijewski J, Thannickal VJ. Novel mechanisms for the antifibrotic action of nintedanib. *Am J Respir Cell Mol Biol* 2016. PMID: 26072676; PubMed Central PMCID: PMC4742925.
3. Bernard K, Logsdon NJ, Ravi S, Xie N, Persons BP, **Rangarajan S**, Zmijewski JW, Mitra K, Liu G, Darley-Usmar VM, Thannickal VJ. Metabolic reprogramming is required for myofibroblast contractility and differentiation. *J Biol Chem* 2015. PMID: 26318453; PubMed Central PMCID: PMC4646190.
4. **Rangarajan S,** Locy ML, Luckhardt TR, Thannickal VJ. Targeted therapy for idiopathic pulmonary fibrosis: Where to now? *Drugs* 2016 Mar;76(3):291-300. PMID: 26729185; PubMed Central PMCID: PMC4939080.
5. Kurundkar AR, Kurundkar D, **Rangarajan S**, Locy ML, Zhou Y, Liu RM, Zmijewski J, Thannickal VJ. The matricellular protein CCN1 enhances TGF-β1/SMAD3-dependent profibrotic signaling in fibroblasts and contributes to fibrogenic responses to lung injury. *FASEB J* 2016. PMID: 26884454; PubMed Central PMCID: PMC4871800.

6. Surolia R, Li FJ, Wang Z, Li H, Liu G, Zhou Y, Luckhardt T, Bae S, Liu RM, **Rangarajan S**, de Andrade J, Thannickal VJ, Antony VB. 3D pulmospheres serve as a personalized and predictive multicellular model for assessment of antifibrotic drugs. *JCI Insight* 2017. PMID: 28138565; PubMed Central PMCID: PMC5256136.
7. **Rangarajan S**, Bernard K, Thannickal VJ. Mitochondrial Dysfunction in Pulmonary Fibrosis. *Ann Am Thorac Soc* 2017. PMID: 29161077 PMCID: PMC5711275.
8. **Rangarajan S**, Bone NB, Zmijewska AA, Jiang S, Park DW, Bernard K, Locy ML, Ravi S, Deshane J, Mannon RB, Abraham E, Darley-Usmar V, Thannickal VJ, Zmijewski JW. Metformin reverses established lung fibrosis in a bleomycin model. *Nat Med* 2018. PMID: 29967351; PubMed Central PMCID: PMC6081262.
9. **Rangarajan S**, Lee JS. The TAMing of the idiopathic pulmonary fibrosis myofibroblast: One step closer? *Am J Resp Crit Care Me*d 2018. PMID: 29664671.

Professional Development: Dr. Rangarajan has presented his work at national meetings of the American Thoracic Society (ATS) and Keystone Symposia on Cellular and Molecular Biology. After completion of his T32 training, Dr. Rangarajan obtained a K08-NIH award (HL135399; 3/1/17-1/28/22). In 2018, he was recruited to the University of Colorado where he is currently an Assistant Professor of Medicine and maintains an active research program.

Name: GENO, Kimball A., Ph.D.   **Appointment date:** 7/1/14 – 6/30/17
Mentor: Moon H. Nahm, Ph.D.; Mark Dransfield, M.D. (Co-Mentor)
Project Title: Defects in Ficolin-2 in Innate Immunity against Pneumoccous

Primary Research Project: We are studying the interactions of the innate immune opsonin ficolin-2 with serotype 11A pneumococcus, a serotype that predominantly and disproportionately afflicts the elderly. We initially discovered inhibitors of ficolin-2 in the archived sera of elderly adults but were unable to replicate this inhibition in freshly collected sera from elderly adults, including donor matched specimens from the archived samples, and we were thus forced to conclude that this inhibition was an artifact of storage; we published these findings late last year. We are currently collecting specimens to evaluate other facets of ficolin-2 function that may become dysfunctional with age, including serum protease concentrations and complement function in general. In related studies, we uncovered the genetic basis differentiating two closely-related serotypes that interact with ficolin-2 (recently submitted), we discovered a novel serotype within serotype 35B that appears to have evolved as a ficolin-2 escape mutant, we developed a new method for the purification of recombinant ficolin-2, and we discovered that commercial processes to remove complement components from sera have the unintended consequence of removing ficolin-2 in these products. I also assisted the laboratory in completion of two studies of serogroup 6 pneumococci and published a review of pneumococcal serotypes with an international coalition of leaders in the pneumococcal field.

Publications (during and as a result of T32-initiated research and training):
1. Brady AM, **Geno KA**, Dalecki AG, Cheng X, Nahm MH. Commercially available complement component-depleted sera are unexpectedly codeplete of ficolin-2. *Clin Vaccine Immuno*l 2014. PMID: 25030054
2. Park IH*, **Geno KA***, Yu J, Oliver MB, Kim KH, Nahm MH. Genetic, biochemical, and serological characterization of a new pneumococcal serotype, 6H, and generation of a pneumococcal strain producing three different capsular repeat units. *Clin Vaccine Immunol* 2015. PMID: 25589550
3. **Geno KA**, Spencer BL, and Nahm MH. Rapid and efficient purification of ficolin-2 using a disposable CELLine bioreactor. *J Immunol Methods* 2015. PMID: 26021447
4. **Geno KA**, Gilbert GL, Hinds J, Jones C, Klugman KP, Konradsen H, Skovsted IC, Song JY, Nahm MH. Pneumococcal capsules and their types:Past, present, future. *Clin Microbiol Rev* 2015.PMID:26085553
5. Burton RL, **Geno KA**, Saad JS, and Nahm MH. Pneumococcus with the "6E" cps locus produces serotype 6B capsular polysaccharide. *J Clin Microbiol* 2015. PMID: 26818670
6. **Geno KA**, Saad JS, and Nahm MH. Discovery of novel pneumococcal serotype 35D, a natural WciG-deficient variant of serotype 35B. *J Clin Microbiol* 2017. PMID:28202800

7. **Geno KA**, Bush CA, Wang M, Jin C, Nahm MH, Yang J. WciG O-acetyltransferase functionality differentiates pneumococcal serotypes 35C and 42. J *Clin Microbio*l 2017. PMID:28659323

Professional Development: Dr. Geno was productive trainee who presented his research at national meetings and at local trainee symposia. After completing his training, Dr. Geno wished to pursue studies in Clinical Chemistry building on his research experience as T32 trainee, and is an Instructor in Pathology and Laboratory Medicine at Dartmouth University.

Name: GUIMBELLOT, Jennifer, M.D., Ph.D.   **Appointment Date:** 8/1/15 – 7/31/16
Mentor: Steven M. Rowe, MD; Amit Gaggar, M.D., Ph.D. (Co-Mentor)
Project Title: Development of Personalized Approaches to Cystic Fibrosis

Primary Research Project: Dr. Guimbellot's studies are focused on investigating a novel three-dimensional nasal epithelial model in suspension for assessment of CFTR function. She has initiated a collaboration with a biotech firm in Huntsville called Synvivo using a microfluidics platform for respiratory epithelial culture, established the model system in her laboratory, and is working on developing a fully differentiated three-dimensional model in a microfluidics chip that, when paired with microOCT, allows simultaneous assessment of multiple parameters of mucociliary clearance and CFTR function. She has also started to establish sweat gland epithelial culture in the laboratory in order to assess differential regulation of CFTR in that tissue compared to respiratory tissue and explore the potential of this tissue type in personalized medicine for CF subjects.

Publications (during and as a result of T32-initiated research and training):
1. **Guimbellot J**, Sharma J, Rowe SM. Toward inclusive therapy with CFTR modulators: Progress and challenges. *Pediatric Pulmonology* 2017. PMID: 28881097; PubMed Central PMCID: PMC6208153.
2. **Guimbellot JS**, Leach JM, Chaudhry IG, Quinney NL, Boyles SE, Chua M, Aban I,Jaspers I, Gentzsch M. Nasospheroids permit measurements of CFTR-dependent fluid transport. *JCI Insight* 2017. PMID: 29202459; PubMed Central PMCID: PMC5752372.
3. **Guimbellot JS**, Acosta EP, Rowe SM. Sensitivity of ivacaftor to drug-drug interactions with rifampin, a cytochrome P450 3A4 inducer. *Pediatric Pulmonology* 2018. PMID:29488691; PubMed Central PMCID: PMC6208148.
4. **Guimbellot J**, Solomon GM, Baines A, Heltshe SL, VanDalfsen J, Joseloff E, Sagel SD, Rowe SM, GOALe(2) Investigators. Effectiveness of ivacaftor in cystic fibrosis patients with non-G551D gating mutations. *J Cyst Fibrosis* 2018. PMID: 29685811; PubMed Central PMCID: PMC6196121.
5. Poore TS, Virella-Lowell I, **Guimbellot JS**. Potential pathogenicity of Inquilinus limosus in a pediatric patient with cystic fibrosis. *Pediatric Pulmonology* 2018. PMID: 29790674; PubMed Central PMCID: PMC6219391.
6. Liu Z, Mackay S, Gordon DM, Anderson JD, Haithcock DW, Garson CJ, Tearney GJ, Solomon GM, Pant
K, Prabhakarpandian B, Rowe SM, **Guimbellot JS.** Co-cultured microfluidics model of the airway optimized for microscopy and microoptical coherence tomography imaging. *Biomed Opt Express* 2019. PMID:31646055; PubMed Central PMCID: PC6788592.

Professional Development: Dr. Guimbellot's training has included participation in the Genetics and Genomics Clinical Research Immersion course, the Clinical and Translational Science Training Program and the Success in Research at UAB workshop. These courses improved her clinical and translational study skill set. Since completion of her T32 training, Dr. Guimbellot was recruited as an Assistant Professor of Pediatrics and has obtained the prestigious Harry Schwachmann Research Award from the CF Foundation in 2017. Further, she obtained a K-grant in 2019.

Name: LARSON-CASEY, Jennifer, Ph.D.   **Appointment date:** 1/12/15 – 1/11/16
Mentor: A. Brent Carter, M.D.; Veena B. Antony, M.D. (Co-Mentor)
Project Title: Cigarette Smoke Impairs Innate Immunity in Alveolar Macrophages

Primary Research Project: Dr. Larson-Casey's research project is focused on the critical role of lung macrophages in innate immunity and in the development of pulmonary fibrosis. Specifically, while a T32 trainee, she investigated the role of mitophagy in the pathogenesis of pulmonary fibrosis and how lung macrophages regulate other cells within the lung. The support provided by the T32 allowed Dr. Casey to focus her research interests into a field distinct from her mentor. She is currently investigating the role of environmental toxins such as heavy metals that influence lung macrophage innate immunity in pulmonary diseases.

Publications (during and as a result of T32-initiated research and training):

1. Murthy S, **Larson-Casey JL**, Ryan AJ, He C, Kobzik L, Carter AB. Alternative activation of macrophages and pulmonary fibrosis are modulated by scavenger receptor, macrophage receptor with collagenous structure. *FASEB J* 2015. PubMed PMID: 25953850; PubMed Central PMCID: PMC4511206

2. Osborn-Heaford HL, Murthy S, Gu L, **Larson-Casey JL**, Ryan AJ, Shi L, Glogauer M, Neighbors JD, Hohl R, Carter AB. Targeting the isoprenoid pathway to abrogate progression of pulmonary fibrosis. *Free Radical Biol Medicine* 2015. PMID: 25958207; PubMed Central PMCID: PMC4554879.

3. He C, **Larson-Casey JL**, Gu L, Ryan AJ, Murthy S, Carter AB. Cu,Zn-superoxide dismutase-mediated redox regulation of jumonji domain containing 3 modulates macrophage polarization and pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2016. PMID: 26699812; PubMed Central PMCID: PMC4942206.

4. Slusser-Nore A, **Larson-Casey JL**, Zhang R, Zhou XD, Somji S, Garrett SH, Sens DA, Dunlevy JR. SPARC expression is selectively suppressed in tumor initiating urospheres isolated from As+3- and Cd+2-transformed human urothelial cells (UROtsa) stably transfected with SPARC. *PLOS One* 2016. PubMed PMID: 26783756; PubMed Central PMCID: PMC4718619.

5. **Larson-Casey JL**, Deshane JS, Ryan AJ, Thannickal VJ, Carter AB. Macrophage Akt1 kinase-mediated mitophagy modulates apoptosis resistance and pulmonary fibrosis. *Immunity* 2016. PubMed PMID: 26921108; PubMed Central PMCID: PMC4794358.

6. **Larson-Casey JL**, Carter AB. Determination of H(2)O(2) Generation by pHPA assay. *Bio Protocols* 2016. PubMed PMID: 28280752; PubMed Central PMCID: PMC5340185.

7. Gu L, **Larson-Casey JL**, Carter AB. Macrophages utilize the mitochondrial calcium uniporter for profibrotic polarization. *FASEB J* 2017. PubMed PMID:28351840; PubMed Central PMCID: PMC5471522.

8. **Larson-Casey JL**, Gu L, Jackson PL, Briles DE, Hale JY, Blalock JE, Wells JM, Deshane JS, Wang Y, Davis D, Antony VB, Massicano AVF, Lapi SE, Carter AB. Macrophage Rac2 is required to reduce the severity of cigarette smoke-induced pneumonia. *Am J Respir Crit Care Med* 2018. PubMed PMID: 29897791; PubMed Central PMCID: PMC6290940.

9. He C, **Larson-Casey** JL, Davis D, Hanumanthu VS, Longhini ALF, Thannickal VJ, Gu L, Carter AB. Nox4 modulates macrophage phenotype and mitochondrial biogenesis in asbestosis. *JCI Insight* 2019. PMID: 31434799; PubMed Central PMCID: PMC6777818.

Professional Development: Dr. Larson-Casey has been highly productive as T32 trainee, and has secured funding from multiple sources to advance her research. She <u>obtained a Parker B. Francis Fellowship in 2017</u> to investigate the role of cadmium in cigarette smoke in host defense against community-acquired respiratory pathogens. She <u>received a research grant from the American Lung Association</u> to study the effects of cigarette smoke on the severity of hospital-acquired pneumonia, and an <u>intramural Faculty Development Grant Program award</u> to study the effects of cadmium from cigarette smoke on intermediates of the mevalonate pathway. Dr. Larson-Casey was <u>promoted to Assistant Professor of Medicine in PACCM Division at UAB in 2017</u>. She has authored several manuscripts and <u>submitted an RO1 on Oct 2019</u>.

Name: GENSCHMER, Kristopher, Ph.D.   **Appointment Date:** 06/01/15 – 05/31/18
Mentor: J. Edwin Blalock, Ph.D.; Amit Gaggar, M.D., Ph.D. (Co-Mentor)
Project Title: Neutrophil Derived Exosomes in the COPD Disease Phenotype

Primary Research Project: Dr. Genschmer's research focuses on investigating biology of proteases which are found on PMN-derived exosomes. Specifically, in previous work in a manuscript in *Cell*, Dr. Genschmer

found that the serine protease neutrophil elastase is found on the surface of PMN-derived exosomes and is capable of hydrolyzing collagen and elastin. Dr. Genschmer's work focuses on the mechanisms which bind neutrophil elastase to exosome surfaces and modulating this interaction in order to better therapeutically target this entity.

Publications (during and as a result of T32-initiated research and training):
1. Oh JY, Hamm J, Xu X, **Genschmer K**, Zhong M, Lebensburger J, Marques MB, Kerby J, Pittet JF, Gaggar A, Patel RP. Absorbance and redox based approaches for measuring free heme and free hemoglobin in biological matrices. *Redox Biology* 2016. PMID: 27566280; PubMed Central PMCID: PMC5007433.
2. O'Reilly PJ, Ding Q, Akthar S, Cai G, Genschmer KR, Patel DF, Jackson PL, Viera L, Roda M, Locy ML, Bernstein EA, Lloyd CM, Bernstein KE, Snelgrove RJ, Blalock JE. Angiotensin-converting enzyme defines matrikine-regulated inflammation and fibrosis. *JCI Insight* 2017. PMID: 29202450; PubMed Central PMCID: PMC5752376.
3. Lal CV, Olave N, Travers C, Rezonzew G, Dolma K, Simpson A, Halloran B, Aghai Z, Das P, Sharma N, Xu X, **Genschmer K**, Russell D, Szul T, Yi N, Blalock JE, Gaggar A, Bhandari V, Ambalavanan N. Exosomal microRNA predicts and protects against severe bronchopulmonary dysplasia in extremely premature infants, *JCI Insight* 2018. PMID: 29515035; PubMed Central PMCID: PMC5922295.
4. **Genschmer KR*,** Russell DW*, Lal CV, Szul T, Bratcher PE, Noerager BD, Abdul Roda M, Xu X, Rezonzew G, Viera L, Dobosh BS, Margaroli C, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, Tirouvanziam R, Gaggar A*, Blalock JE*. Activated PMN exosomes: Pathogenic entities causing matrix destruction and disease in the dung, *Cell* 2019, PMID: 30633902.

Professional Development: Dr. Genschmer attended the American Society of Exosomes and Microvesicles 10/2017, where he presented a research talk as well as a related poster. In 2017, he attended the American Thoracic Society where he presented a poster at the RAPID poster discussion session. He also attends weekly Interstitial Lung Disease conferences, as well as Pulmonary Grand Rounds, in addition to monthly meetings of the Pulmonary and Critical Care Journal Club, Pulmonary case meetings and Research in Progress. He has been extremely productive, with a recent first-author publication in the journal, *Cell*. Dr. Genschmer transitioned from the T32, effective 6/20/18, to an NIH R35 (PI: Ed Blalock) as a Co-Investigator. He was appointed as an Assistant Professor of Medicine in the Division of Pulmonary, Allergy, and Critical Care Medicine at UAB, effective, 7/01/18. He had his initial submission of his first RO1 in 2019 and will be resubmitting in early 2020.

Name: CURTISS, Miranda, M.D., Ph.D.   **Appointment Date:** 02/01/16 – 9/30/17
Mentor: Frances Lund, Ph.D.; Jessy Deshane (Co-Mentor)
Project Title: Dendritic Cell Subsets in Th2 Cell Polarization

Primary Research Project: Dr. Curtiss is studying the transcriptional profile of dendritic cells that polarize Th2 cells. She has characterized the transcriptional differences between migratory dendritic cell subsets in the gut draining lymph node (CD11b+ vs. CD103+) in *H polygyrus* infected mice using RNA seq, and compared these to the transcriptomes of dendritic cells genetically deficient in CXCR5. She isolated these cells from mixed bone marrow radiation chimeras, which permit the generation of a normal immune response to *H polygyrus* by wildtype cells that provide a foil for the CXCR5 knockout dendritic cells. The direct comparison of wildtype and CXCR5 knockout dendritic cells from the same animal minimizes experimental variability, providing a robust data set for further analysis. The secreted protein, Chi3L1 (the mouse homolog of YKL-40), was differentially regulated between CD11b+ and CD103+ DC subsets, and between CXCR5 expressing and deficient CD11b+ DCs. YKL-40 has been implicated in asthma development and severity in patients, and its homologue is similarly important in mouse models of allergic airway disease. However, the majority of this biologic effect is thought to be mediated by epithelial cells and M2 macrophages; the importance of Chi3L1 expression in dendritic cells is novel. Dr. Curtiss has identified gene sets that are upregulated and downregulated in response to CXCR5, and genes which are coordinately and differently regulated between DC

subsets. To confirm the importance of these genes in Th2 polarization of T cells, she has collaborated to establish a new model in the Lund lab using enteric infection with *Citrobacter rodentii* to establish a Th17 mediated infection, uncovering new pathways Th2/Th17 polarization.

Publications (during and as a result of T32-initiated research and training):
1. Hough KP, Wilson LS, Trevor JL, Strenkowski JG, Maina N, Kim YI, Spell ML, Wang Y, Chanda D, Dager JR, Sharma NS, **Curtiss M**, Antony VB, Dransfield MT, Chaplin DD, Steele C, Barnes S, Duncan SR, Prasain JK, Thannickal VJ, Deshane JS. Unique lipid signatures of extracellular vesicles from the airways of asthmatics. *Sci Reports* 2018. PMID: 29985427
2. **Curtiss M**, Ballesteros N, Rosenberg A, Ptacek T, Leon B, Ballesteros-Tato A, Steele C, Randall T, Lund F. Chi3l1 expression by dendritic cells regulates Th2 responses. To be submitted to Journal of Immunology.

Professional Development: Dr. Curtiss successfully transitioned from the T32 and was awarded the Walter B. Frommeyer award from the Department of Medicine and a Dixon Fellowship Award from the Department of Pediatrics.   At the same time, she was appointed as an Assistant Professor of Medicine in the Division of Pulmonary, Allergy and Critical Care at UAB. She is planning a K08 submission in 2020, focusing on chitinase and dendritic cell signaling in asthma.

Name: RUSSELL, Derek, M.D.   **Appointment Date:** 07/01/17 – 06/30/18
Mentor: J. Edwin Blalock, Ph.D.; Amit Gaggar, M.D., Ph.D. (Co-Mentor)
Project Title: Proteolytic Neutrophil Exosomes in COPD

Primary Research Project: Dr. Russell's research has focused on exosomes released from activated neutrophils which express proteolytically active neutrophil elastase (NE). Using *in vitro* enzymologic and *in vivo* murine exposure models, he has linked the NE+ PMN derived exosome to COPD pathogenesis, finding that they bind directly to NE substrates in the lung, such as type I collagen and elastin via an integrin (Mac-1), and are resistant to the antiprotease alpha-1 antitrypsin. He has also elucidated the binding mechanism of NE to the exosome surface. In vivo, Dr. Russell found that these exosomes cause alveolar enlargement, RV hypertrophy, and spirometric obstruction when delivered intratracheally to mice. He has studied the analogous population of neutrophil derived exosomes in bronchoalveolar lavage fluid of COPD subjects and recapitulated these in vitro findings. The findings of this project have recently been published in a manuscript at *Cell* (co-first author).   Dr. Russell's project will focus on phenytyping COPD subjects with high exosome burden and to determine if these particles may serve as biomarkers which predict loss of lung function over time. He also continues to conduct pragmatic clinical trials within the ICU.

Publications (during and as a result of T32-initiated research and training):
1. Semler MW, Janz DR, **Russell DW**, Casey JD, Lentz RJ, Zouk AN, deBoisblanc BP, Santanilla JI, Khan YA, Joffe AM, Stigler WS, Rice TW; Check-UP Investigators(∗); Pragmatic critical care research group. A multicenter, randomized trial of ramped position vs. sniffing position during endotracheal intubation of critically ill adults. *Chest* 2017. PMID: 28487139; PubMed Central PMCID: PMC5812765.
2. McNeil JB, Shaver CM, Kerchberger VE, **Russell DW**, Grove BS, Warren MA, Wickersham NE, Ware LB, McDonald WH, Bastarache JA. Novel method for noninvasive sampling of the distal airspace in Acute Respiratory Distress Syndrome. *Am J. Respir Crit Care Med* 2018.PMID: 29253353; PubMed Central PMCID: PMC5909163.
3. Lal CV, Olave N, Travers C, Rezonzew G, Dolma K, Simpson A, Halloran B, Aghai Z, Das P, Sharma N, Xu X, Genschmer K, **Russell D**, Szul T, Yi N, Blalock JE, Gaggar A, Bhandari V, Ambalavanan N. Exosomal microRNA predicts and protects against severe bronchopulmonary dysplasia in extremely premature infants. *JCI Insight* 2018. PMID: 29515035; PubMed Central PMCID: PMC5922295.
4. Casey JD, Janz DR, **Russell DW**, Vonderhaar DJ, Joffe AM, Dischert KM, Brown RM, Lester MG, Zouk AN, Gulati S, Stigler WS, Rice TW, Semler MW; PreVent Investigators and the Pragmatic Critical Care Research Group. Manual ventilation to prevent hypoxaemia during endotracheal intubation of critically ill adults: protocol and statistical analysis plan for a multicentre randomized trial. *BMJ Open* 2018. PMID:30099400; PubMed Central PMCID: PMC6089322.

5. Genschmer KR*, **Russell DW***, Lal CV, Szul T, Bratcher PE, Noerager BD, Abdul Roda M, Xu X, Rezonzew G, Viera L, Dobosh BS, Margaroli C, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, Tirouvanziam R, Gaggar A*, Blalock JE*. Activated PMN exosomes: Pathogenic entities causing matrix destruction and disease in the lung. *Cell* 2019. PMID: 30633902.
6. **Russell DW**, Hardison M, Genschmer KR, Szul T, Bratcher PE, Abdul Roda M, Xu X, Viera L, Blalock JE, Gaggar A, Noerager BD. Benzoxylcarbonyl-proline-prolinal (ZPP): Dual complementary roles for neutrophil Inhibition. *Biochem Biophys Res Comm* 2019. PMID: 31400851; Pubmed Central PMCID: PMC6730661.
7. Janz DR, Casey JD, Semler MW, **Russell DW**, Dargin J, Vonderhaar DJ, Dischert KM, West JR, Stempek S, Wozniak J, Caputo N, Heideman BE, Zouk AN, Gulati S, Stigler WS, Bentov I, Joffe AM, Rice TW. Effect of a fluid bolus on cardiovascular collapse amongst critically ill adults undergoing tracheal intubation (PrePARE): a randomized controlled trial. *Lancet Respiratory Med* 2019. PMID: 31585796.

Professional Development: Since joining the T32, Dr. Russell has coauthored seven peer review manuscripts accepted or published, including *Cell* (co-first author)*, American Journal of Respiratory and Critical Care Medicine, Lancet Resp Medicine*, *JCI Insight*, and *Chest*. He has presented his research talks at the American Society of Exosomes and Microvesicles in October, 2017, and at the international meeting of the American Thoracic Society (ATS) in May 2018; for the latter, he received a Trainee Research Award from the ATS. Dr. Russell is the site-PI of two ongoing randomized clinical trials in the medical intensive care unit (MICU), has six total papers submitted to peer reviewed journals that are in pre- acceptance phases. He regularly attends all didactic seminar series of the pulmonary division including Journal Clubs, Research in Progress and Pulmonary Grand Rounds. He moderates the weekly MICU mortality review as well as a monthly educational lecture to the MICU house staff on the topic of evidenced-based medicine. He has taken an active role in clinical trials and ICU-based research at the Birmingham VA Medical Center. He was also appointed as an Assistant Professor of Medicine in the Division of Pulmonary, Allergy and Critical Care at UAB in July, 2018. Finally, he has submitted a K08 grant in 2019 which has received a fundable score.

Name: PAREKH, Trisha, D.O. **Appointment Date:** 08/01/16 – 07/31/19
Mentor: Mark Dransfield, M.D.; Smita Bhatia, M.D. (Co-Mentor)
Project Title: Social Determinants of COPD Progression and Outcomes

Primary Research Project: Dr. Parekh's research focuses on the social determinants of health, specifically COPD-related hospitalizations and outcomes. She is collecting primary data on COPD patients at the Kirklin Clinic and at the Lung Health Center. She has collected 142 out of 150 surveys for her database that includes over 100 variables from which she will analyze the impact of specific social determinants on health of COPD patients. Dr. Parekh submitted a proposal to SPIROMICS which was recently accepted that will evaluate the relationship between inflammatory biomarkers, low socioeconomic status, and outcomes in COPD.

Presentations/Publications (during fellowship training; T32 appointment):
1. **Parekh TM**, Bhatt SP, Westfall AO, Wells JM, Kirkpatrick D, Iyer AS, Mugavero M, Willig JH, Dransfield MT. Implications of DRG classification in a bundled payment initiative for COPD. *Am J Accountable Care* 2017. PMID: 29623307; PubMed Central PMCID: PMC5881946.
2. Bhatt SP, Wells JM, Iyer AS, Kirkpatrick DP, **Parekh TM**, Leach LT, Anderson EM, Sanders JG, Nichols JK, Blackburn CC, Dransfield MT. Results of a Medicare Bundled Payments for Care Improvement Initiative for Chronic Obstructive Pulmonary Disease Readmissions. *Ann Am Thoracic Society* 2017. PMID: 28005410; PubMed Central PMCID: PMC5802596.
3. **Parekh TM**, Wu C, McClure LA, Howard VJ, Cushman M, Malek AM, Harrington KF, Cherrington AL, Dransfield MT, Bhatia S. Determinants of cigarette smoking status in a national cohort of black and white adult ever smokers in the USA: a cross-sectional analysis of the REGARDS study. *BMC Open* 2019. PMID: 31079085; PubMed Central PMCID: PMC6530531.
4. **Parekh TM**, Copeland CR, Dransfield MT, Cherrington A. Application of the community health worker model in adult asthma and COPD in the US: A systematic review. *BMC Pulmonary* 2019. PubMed PMID: 31242944; PubMed Central PMCID: PMC6593583.

5. **Parekh TM**, Bhatia S, Cherrington A, Kim YI, Lambert A, Iyer A, Regan EA, DeMeo DL, Han M, Dransfield MT. Factors influencing decline in the quality of life in smokers without airflow obstruction: The COPDGene study. *Respiratory Med* 2019. PMID: 31759270

Professional Development: Dr. Parekh was accepted into the Clinical Investigator's Training Program which she has since completed. Dr. Parekh was also accepted as a Health Disparities Research Scholar. This year-long training program with weekly classes, regular writing retreats, and a preparation of a grant proposal provided valuable information for continued career development. She is enrolled in the MSPH degree with a focus in outcomes research. She presented her latest research at the Health Disparities Symposium 03/2018 as an oral presentation and the American Thoracic Society conference in a Rapid Poster Discussion in 05/2018. She also gave an expert talk on the "Social Determinants of Health-Implications for Pulmonary Medicine" at the 2018 ATS International Conference. Dr. Parekh meets with her panel of mentors on a bimonthly or monthly basis. <u>She is currently an Instructor in Medicine, Division of Pulmonary, Allergy, and Critical Care</u>. She has obtained a Walter B. Frommeyer Grant award this year from the UAB Department of Medicine and plans to submit an NIH K23 application in 2020.

Name: SHEI, Ren-Jay, Ph.D.　　　　　　　　　　　**Appointment Date:** 11/01/16 – 08/02/2019
Mentor: Steven Rowe, M.D.; David Bedwell, Ph.D. (Co-Mentor)
Project Title: Novel Therapeutics in Cystic Fibrosis Lung Disease

Primary Research Project: Dr. Shei's research involves developing novel strategies for treatment of airways disease characterized by impaired mucociliary clearance. He is developing mRNA replacement therapies delivered via biodegradable nano-particles; anti-sense oligonucleotide treatment therapy; RNAi therapy for cystic fibrosis; novel glycopolymer treatment in ameliorating mucus defects induced by cigarette smoke and in muco-obstructive diseases; and characterizing phenotypic differences in skeletal muscle in patients with cystic fibrosis. Further, based on preliminary data from the RNAi therapy project, a new project to investigate novel biology between CFTR and ENaC proteins is now underway. This project will aim to understand interactions that take place between CFTR and ENaC, in particular, whether ENaC modulates CFTR function.

Presentations/Publications (during fellowship training; T32 appointment):
1. Brand JD, Lazrak A, Trombley JE, **Shei RJ**, Adewale AT, Tipper JL, Yu Z, Ashtekar AR, Rowe SM, Matalon S, Harrod KS. Influenza-mediated reduction of lung epithelial ion channel activity leads to dysregulated pulmonary fluid homeostasis. *JCI Insight* 2018. PMID: 30333319; PubMed Central PMCID: PMC6237450
2. **Shei RJ**, Peabody JE, Kaza N, Rowe SM. The epithelial sodium channel (ENaC) as a therapeutic target for cystic fibrosis. *Curr Opin Pharmacology* 2018. PMID: 30340955; PMCID: PMC6294660.
3. **Shei RJ**, Peabody JE, Rowe SM. Functional anatomical imaging of the airway surface. *Ann Am Thor Soc* 2018. PMID:30431349; PubMed Central PMCID: PMC6322031.
4. Leung HM, Birket SE, Hyun C, Ford TN, Cui D, Solomon GM, **Shei RJ**, Adewale AT, Lenzie AR, Fernandez-Petty CM, Zheng H, Palermo JH, Cho DY, Woodworth BA, Yonker LM, Hurley BP, Rowe SM, Tearney GJ. Intranasal micro-optical coherence tomography imaging for cystic fibrosis studies. *Science Translational Med* 2019. PubMed PMID: 31391319

Professional Development: Dr. Shei attended and presented at the 2017 and 2018 American Thoracic Society and the North American Cystic Fibrosis conferences. Dr. Shei has published four papers, and has greatly contributed to our knowledge of CF airway anatomy. He currently works as a medical science liaison for Mallinckrodt, Inc.

<u>**CURRENT TRAINEES:**</u>
Name: HE, Chao, M.D., Ph.D.　　　　　　　　　**Appointment Date:** 03/01/18 – present
Mentor: A. Brent Carter, M.D.; Victor J. Thannickal, M.D. (Co-Mentor)
Project Title: NOX4 Modulates Lung Macrophage Polarization in Pulmonary Fibrosis

Primary Research Project: Dr. He's research is focused on defining the role of alveolar macrophages in lung fibrosis. He has previously shown that macrophages undergo different polarization process during fibrosis development and the process is regulated by redox signaling. His current work investigates NOX4 and its role in mediating lung macrophage polarization. Preliminary data from this work showed NOX4 promotes lung macrophages polarized into a pro-fibrotic phenotype via modulating mitochondrial biogenesis. He is working on generating a macrophage/monocyte specific NOX4 knock-out mice model to evaluate the role of lung macrophages in different pulmonary diseases.

Presentations/Publications (during fellowship training; T32 appointment):
1. **He C**, Larson-Casey JL, Davis D, Hanumanthu VS, Longhini ALF, Thannickal VJ, Gu L, Carter AB. Nox4 modulates macrophage phenotype and mitochondrial biogenesis in asbestosis. JCI Insight 2019. PMID: 31434799; PubMed Central PMCID: PMC6777818.
2. Larson-Casey JL, Vaid M, Gu L, **He C**, Cai GQ, Ding Q, Davis D, Berryhill TF, Wilson LS, Barnes S, Neighbors JD, Hohl RJ, Zimmerman KA, Yoder BK, Longhini ALF, Hanumanthu VS, Surolia R, Antony VB, Carter AB. Increased flux through the melvalonate pathway mediates fibrotic repair without injury. *J of Clinical Investigation* 2019. PMID: 31609245; PubMed Central PMCID: PMC6819144.

Professional Development: Dr. He is currently in his final (fifth) year of ABIM Research Pathway (Physician-Scientist Training Program) and nearing the completion of his first year on the Pulmonary T32. He attends Pulmonary Grand Rounds, Journal Clubs and other fellowship conferences routinely to complement his research. Dr. He presented at the 2018 American Association of Physician/American Society of Clinical Investigator Joint Meeting in April of 2018 and the 2018 and 2019 American Thoracic Society International Conferences. He plans on submitting a grant proposal for the Parker B. Francis Fellowship in 2020 and has also submitted his K08 grant and is currently awaiting scoring.

Name: LAFON, David C., M.D.   **Appointment date:** 07/01/18 – present
Mentor: Moon H. Nahm MD; Mark T. Dransfield (Co-Mentor)
Project Title: Immune Responses to Pneumococcal Vaccines

Primary Research Project: Dr. LaFon's research is focused on measuring immune responses to pneumococcal vaccines in order to characterize the immune dysfunction associated with COPD. Since current methods for diagnosing immune dysfunction are inadequate, Dr. LaFon is interested in identifying novel means of evaluating vaccine responses in clinical populations, and applying these techniques to COPD. In particular, he plans to investigate the use of multiplexed opsonophagocytosis assays (MOPA) as an indicator of pneumococcal antibody function in individuals with COPD who experience frequent infections. He is working with Dr. Mark Dransfield to apply analytical techniques used in the Nahm Lab to study clinical populations. Dr. LaFon is currently a co-investigator in a multi-site study of immune parameters in COPD. He is also analyzing vaccine response data in COPD and initiating a study of pneumococcal antibodies in COPD sera. Dr. LaFon conducted a study demonstrating that different laboratories' assays that measure pneumococcal antibodies generate different results, with the potential to affect clinical diagnosis. He is first-author on a manuscript describing these findings which has been accepted to the *Journal of Allergy and Clinical Immunology*.
Presentations/Publications (during fellowship training; T32 appointment):
1. **LaFon DC**, Nahm MH. Measuring quantity and function of pneumococcal antibodies in immunoglobulin products. *Transfusion* 2018. PMID: 30536435
2. **LaFon DC**, Nahm M. Inter-laboratory variability in multiplexed pneumococcal antibody testing. *J Allergy Clin Immunology* 2018. PubMed PMID: 30468776.
3. **LaFon DC**, Nahm MH. Measuring immune responses to pneumococcal vaccines. *J Immunol Methods* 2018. PMID:30098317; PubMed Central PMCID: PMC6169307.
4. **LaFon DC**, Bhatt SP, Labaki WW, Rahaghi FN, Moll M, Bowler RP, Regan EA, Make BJ, Crapo JD, San Jose Estepar R, Diaz AA, Silverman EK, Han MK, Hobbs B, Cho MH, Washko GR, Dransfield MT, Wells JM. Pulmonary artery enlargement and mortality risk in moderate to severe COPD: results from COPDGene. *Eur Resp J* 2019, PMID: 31772001; PubMed Central PMCID: PMC5812765.

Professional Development: Since joining the T32, Dr. LaFon has been first author on three peer-reviewed manuscripts written with Dr. Nahm that have been accepted or published. In addition to the manuscript accepted to *Journal of Allergy and Clinical Immunology*, one manuscript has been published in *Journal of Immunological Methods*, and another accepted for publication in *Transfusion*. He regularly attends pulmonary division didactic lectures and conferences. He is planning a K23 submission in middle of 2020.

Name: GOLIWAS, Kayla, Ph.D.   **Appointment Date:** 07/01/18 – present
Mentor: Jessy Deshane, Ph.D.; Victor J. Thannickal, M.D. (Co-Mentor)
Project Title: Tissue Engineered Three-Dimensional Models of Lung Disease

Primary Research Project: Dr. Goliwas' research is focused on generating representative *in vitro* models of lung carcinoma that include components of the tumor microenvironment, namely immune cell subsets and fibroblasts within a volume of extracellular matrix, as currently no *in vitro* tumor models that are considered immune competent exist. The function of the immune system within the tumor microenvironment is complex, with accumulating data indicating a cancer-promoting effect in many established tumors as tumor cells are able to evade immune detection through multiple mechanisms. In recent years, modulation of the patients' immune system has been utilized as a treatment strategy with some success, yet many patients do not respond or are not eligible for current immune targeted therapies. Combining methods within biomedical engineering, cell biology, and tumor immunology, Dr. Goliwas will develop novel models, or tumor mimics, to better recapitulate human malignancy. Real-time changes in the immune cell phenotype and exosome profile within the engineered tumor mimics will be evaluated to monitor how these changes influence T cell exhaustion with and without immune targeted therapy, providing human-specific data to answer a complex question that are difficult to address clinically. These mimics will allow for better understanding of tumor-immune cell cross talk and efficacy of immunotherapy. The proposed tumor mimics have the potential to be utilized in studies involving tumor biology, drug development and personalized medicine, providing an innovative way to study possible changes to the immune milieu *in vitro*. These tumor mimics have the potential to elucidate changes in tumor-immune interactions, and thereby response to immunotherapy, in both a cancer mutation-status/subtype-specific and potentially patient-specific manner.

Presentations/Publications (during fellowship training; T32 appointment):
1. Wang Y, **Goliwas KF**, Severino PE, Hough KP, Van Vessem D, Wang H, Koomulli RP, Frost AR, Ponnazhagen S, Berry JL, and Deshane JS. Mechanical strain induces phenotypic changes in breast cancer cells and promotes immunosuppression in the tumor microenvironment. *in revision for Molecular Cancer Research*.
2. Hough KP, Trevor JL, Chacko BK, Strenkowski JG, Wang Y, **Goliwas KF**, Bone NB, Vang S, Bodduluri S, Steele C, Tousif S, Chin J, Chanda D, Zmijewski J, Antony VB, Dokland T, Mitra K, Zhang J, Duncan SR, Thannickal VJ, Gabrielsson S, Darley-Usmar VM, and Deshane JS. Exosomal transfer of mitochondria and T helper polarization in asthmatics. *In Preparation for submission to Cell.*
3. **Goliwas KF**, Hough KP, Merritt RK, Ashraf H, Jaskula-Stzul R, Berry JL, and Deshane JS. Tumor-stromal interactions are mediated by extracellular vesicles in engineered lung tumor mimics. *In Preparation for submission to The Journal of Immunology.*
4. Goliwas KF, Wang Y, Berry JL, Donahue JM, and Deshane JS. Patient-derived engineered lung tumor mimics maintain tissue heterogeneity and allow for assessment of tumor-stromal interactions. *In Preparation for submission to Nature Methods.*

Professional Development: Dr. Goliwas was recruited to this T32 Training after recently completing her Ph.D. training in Cellular and Molecular Biology at Vanderbilt University. She has already begun to generate interesting new findings of tumor cell-immune interactions that was presented at the 2019 American Thoracic Society Conference; she is first-author on multiple manuscripts that are in-preparation and is first author on a paper currently in revision. She plans on submitting a Parker B Francis grant in 2020.

Name: ROBESON, Sarah M.D.   **Appointment Date:** 07/01/19- present

Mentor: Amit Gaggar, M.D. Ph.D.; J. Edwin Blalock, Ph.D. (Co-Mentor)
Project Title: Regulation of the Matrikine Proline-Glycine-Proline by Peptide Transporters in Lung Disease

Primary Research Project: Dr. Robeson is currently a second-year fellow in Dr. Gaggar's laboratory, working on a project to study the regulation of the matrikine PGP in the inflamed lung. Her work relates to a novel mechanism involving the transport of this peptide out the lung by the peptide transporter PepT2. This project will provide a translational platform for the development of new approaches to the treatment of chronic inflammation. Her first-authored manuscript related to this work has been submitted for publication.

Presentations/Publications (during fellowship training; T32 appointment):
Robeson SW, Margaroli C, Li Jindong, Snelgrove R, Blackburn JP, Blalock JE, Xu X, Gaggar A. A novel mechanism of active matrikine regulation in acute pulmonary inflammation. Submitted to *Journal of Clinical Investigation*.

Professional Development: Based on Dr. Robeson's exemplary research performance during the second year of her fellowship, we have committed to recruiting her to our Pulmonary T32 Training Program, starting at the beginning of her third year in the fellowship program (July, 2019). She presented her research at the 2019 International American Thoracic Society meeting in Dallas, TX and will also present in the 2020 ATS Meeting. We anticipate that Dr. Robeson will join the faculty in August 2020 as an Instructor physician-scientist in the Pulmonary division at UAB.