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TITLE: Idiopathic Pulmonary Fibrosis, a Disease Initiated by Mucociliary Dysfunction

PRINCIPAL INVESTIGATOR: David A. Schwartz, MD

CONTRACTING ORGANIZATION: Regents of the University of Colorado
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## 14. ABSTRACT

The overarching goal of this Program is to develop the scientific knowledge needed to predict and prevent the progression of IPF. We postulate that IPF is caused by recurrent injury/repair/regeneration at the bronchoalveolar junction secondary to overexpression of MUC5B, mucociliary dysfunction, retention of particles, ER stress, and disruption of normal reparative and regenerative mechanisms in the distal lung. During the first year of funding, we have (1) obtained local and DoD approvals for human and animal research; (2) enrolled 26 first degree relatives of individuals with IPF and completed all study procedures for Project 1; (3) performed ChIP, MNase, and TF binding assays to show that MUC5B promoter region is hyperchippable and that HIF1 and GCF bind in this region (Project 2); (4) imported and bred new strains of mice (St3gal3, Fut2, Ern2, Ift88, and Arl13b) in Projects 3 and 4; (5) developed and assessed the amounts and glycosylation of Muc5b in mouse models at baseline, and identified changes in polymer size and migration after inflammatory challenge (Project 3); (6) identified 10 weeks post-injury as a key timepoint for increased ciliogenesis in Muc5b Tg mice and began characterization of ciliogenesis in human lung, and (7) presented findings at two international conferences and published two manuscripts.

**15. SUBJECT TERMS**
preclinical pulmonary fibrosis, biomarkers, airway mucin, mucin 5b polymer, mucociliary dysfunction, transcriptional regulation, lung repair, lung regeneration, ER stress, ciliogenesis

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# Annual Technical Progress Report
## Reporting period: 9/30/17-9/29/18

# 1. Introduction

**The overarching challenge of this Program is to develop the scientific knowledge needed to predict and prevent the progression of Idiopathic Pulmonary Fibrosis (IPF).** IPF affects 5 million worldwide, disproportionately affects men, is associated with cigarette smoking and combat-related particulate exposures, increases with age, is inexplicably increasing in prevalence, is a source of morbidity and mortality among military personnel, and is likely underdiagnosed. Patients with IPF are usually diagnosed when the fibroproliferative process has caused permanent and extensive lung parenchymal damage. Given the irreversible nature of this disease, even approved treatments for IPF only modestly slow progression and have not been shown to alter the 3-5 year survival following diagnosis. We have found that: 1) a gain-of-function *MUC5B* promoter variant rs35705950 is the strongest risk factor (genetic and otherwise) for the development of IPF, accounting for at least 30% of the risk of disease; 2) rs35705950 can be used to identify individuals in the preclinical phase of this life-threatening disease; 3) MUC5B represents a key molecule to understand the mechanisms that initiate the fibroproliferative process in the bronchoalveolar epithelium; and 4) focusing on MUC5B may provide a unique opportunity to define the early molecular events that lead to the development of IPF. We propose that a comprehensive, multi-dimensional approach that focuses on MUC5B transcription in airway epithelia, biological consequences of MUC5B overproduction that are mediated by airway epithelia and cilia, and biomarkers to predict preclinical pulmonary fibrosis (PrePF) and identify those at risk of disease progression could conceivably change the approach in IPF from palliative to preemptive.

# 2. Keywords

Preclinical pulmonary fibrosis, biomarkers, airway mucin, mucin 5b polymer, mucociliary dysfunction, transcriptional regulation, lung repair, lung regeneration, ER stress, ciliogenesis

# 3. Accomplishments

## a. What were the major goals of the project?

**pink shading denotes items that were worked on during this project period

| **Specific Aim 1: Screen 500 asymptomatic siblings of sporadic IPF cases and perform pulmonary function testing on cases of preclinical pulmonary fibrosis (PrePF).** | **Timeline Years 1-3** | **Site 1** (David Schwartz) | **Sites 2-6** Brown, Keith, Loyd, Kass, Wolters, de Adrade |
|---|---|---|---|
| **Major Task 1:** Recruitment sporadic IPF siblings. | Years 1-3 | | |
| Subtask 1: Coordinate with the site PIs to obtain IRB approval at each site for this study. | 1-2 mos | Schwartz, Mathai | " " |
| *Milestone #1: Secure IRB approval at all sites for subject recruitment.* | 1-6 mos | Schwartz, Mathai | " " |
| Subtask 2: Coordinate with the Investigators to consent IPF subjects to contact their siblings for study recruitment. | 1-3 years | Schwartz, Mathai, Schwarz, Lee | " " |

| | | | |
|---|---|---|---|
| Subtask 3: Contact siblings of IPF patients to consent them for the study. | 1-3 years | Schwartz, Mathai | |
| **Major Task 2:** Obtain high-resolution computed tomography (HRCT) scans of chest on recruited siblings from Major Task 1. | 1-3 years | Schwartz, Mathai, Schwarz | |
| **Major Task 2:** Obtain expert radiology reads of the CT images with thoracic radiologists. | 1-3 years | Lynch, Mathai | |
| **Major Task 3:** Obtain pulmonary function testing (PFTs) on all subjects who have CT scans that are positive for the presence of PrePF. | 1-3 years | Schwartz, Mathai, Schwarz, Lee | |
| *Milestone #2: Prepare and submit manuscript on the prevalence and radiographic features of PrePF in the siblings of patients with sporadic IPF.* | *2-3 years* | *All investig.* | *All investig.* |
| **Specific Aim 2: Develop and validate a biomarker profile that improves the detection of preclinical pulmonary fibrosis (PrePF).** | **Timeline Years 1-3** | Site 1 (Schwartz) | Sites 2-5 " " |
| **Major Task 1:** Develop biomarker panel in PrePF cases from FIP family members.<br>- Somapanel, plasma MUC5B, mucin-specific glycans<br>- RNA-seq on PBMC RNA, PBMC telomere length<br>- Genotyping for MUC5B promoter polymorphism | 1 year | Schwartz, Mathai, Yang, Fingerlin | |
| **Major Task 2:** Validate biomarker panel in PrePF cases from sporadic IPF family members.<br>- Somapanel, plasma MUC5B, mucin-specific glycans<br>- RNA-seq on PBMC RNA, PBMC telomere length<br>- Genotyping for MUC5B promoter polymorphism | 1-3 years | " " | |
| *Milestone #3: Prepare manuscript on development of peripheral blood biomarker profile of PrePF.* | *3 years* | *All investig.* | *All investig.* |
| **Specific Aim 3: Elucidate the determinants of progression in preclinial pulmonary fibrosis (PrePF).** | **Timeline Years 3-4** | **Site 1** (Schwartz) | **Site 2** " " |
| **Major Task 1:** Recontact all subjects with Year 1-3 scans positive for PrePF and perform repeat HRCT 2-3 years after study enrollment. | Years 3-4 | Schwartz, Mathai, Schwarz, Lee | " " |
| **Major Task 2:** Re-contact subjects with Year 1-3 scans positive for PrePF and perform repeat PFTs 2-3 years after initial study enrollment. | Years 3-4 | " " | " " |
| **Major Task 3:** Recontact all subjects with initial Year 1-3 scans positive for PrePF and perform repeat peripheral blood draw. | Years 3-4 | " " | " " |
| **Major Task 4:** Measure the same peripheral biomarkers in the collected samples as described under Aim 2. | Years 3-4 | Schwartz, Mathai, Yang | |

| | | | |
|---|---|---|---|
| **Major Task 5:** Analyze differences in peripheral blood biomarkers between subjects with PrePF and those without PrePF. | Year 4 | All investig. | |
| *Milestone #4: Prepare manuscript on development of peripheral blood biomarker profile of progressive PrePF.* | *Year 4* | *All investig.* | *All investig.* |

**Projected Quarterly Enrollment**

| | Year 1 | | | | Year 2 | | | |
|---|---|---|---|---|---|---|---|---|
| **Target Enrollment** | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
| UCO | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| DVAMC | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| NJH | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| UPMC | 5 | 5 | 5 | 5 | 10 | 10 | 10 | 10 |
| UCSF | 5 | 5 | 5 | 5 | 10 | 10 | 10 | 10 |
| UAB | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Vanderbilt | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Target Enrollment | 40 | 80 | 120 | 160 | 210 | 260 | 310 | 360 |

| | Year 3 | | | | Year 4 | | | |
|---|---|---|---|---|---|---|---|---|
| **Target Enrollment** | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
| UCO | 5 | 5 | 1 | 1 | - | - | - | - |
| DVAMC | 5 | 5 | 1 | 1 | - | - | - | - |
| NJH | 4 | 4 | 3 | 2 | - | - | - | - |
| UPMC | 15 | 15 | 6 | 5 | - | - | - | - |
| UCSF | 4 | 4 | 2 | 3 | | | | |
| UAB | 5 | 5 | 4 | 4 | | | | |
| Vanderbilt | 10 | 10 | 6 | 5 | - | - | - | - |
| **Target Enrollment (cumulative)** | **408** | **456** | **479** | **500** | **500** | **500** | **500** | **500** |

**Project 2**

Site 1: University of Colorado, National Jewish Medical Center
12700 E 19th Ave, RC2 – 10025
Aurora, CO 80045
Initiating PI: Drs. Sean Colgan and Anthony Gerber

| **Specific Aim 1:** ***Elucidate the molecular regulation of the MUC5B gene promoter relative to identified major IPF-associated polymorphism***. | **Timeline** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) |
|---|---|---|---|
| **Specific Aim 1:** Development of a High-Throughput Imager | Months | | |
| Subtask 1: ***Elucidate transcription factor binding analysis*** Participating teams:<br>• Colgan and Gerber labs will oversee molecular analysis of ChIP assays and luciferase reporter constructs | 1-4 | Colgan Gerber | |
| Subtask 2: Generate MUC5B SNP cell line<br>• Colgan, Gerber & Seibold will work together to oversee the generation of these lines using CRISPR-Cas9 methods | 4-8 | Colgan Seibold Gerber | |
| Subtask 3: Direct transcription factor loss of function approach in cell lines and primary cells.<br>• Molecular approach to knocking down individual transcription factors (Gerber and Colgan)<br>• Work in primary cells (Seibold and Colgan)<br>• Test transcription factor principles in mice with Evans | 8-12 | Colgan Gerber Evans Seibold | |
| *Milestone #1: Co-author manuscript on MUC5B regulation.* | | | |
| **Specific Aim 2:** ***: Determine integrated transcriptional control of MUC5B expression in response to pro-fibrotic signals*** | | | |
| Subtask 1: ***Use ChIP-loop to determine 3 dimensional chromatin architecture and enhancer interactions within the MUC5B locus***<br>• Determine cooperative binding of FOXA2 using ChIP-look analysis | 12-24 | Gerber | |
| Subtask 2: ***Analyze dynamic MU5B enhancer looping and impact of the promoter variant and pro-fibrotic stimuli.***<br>• Analyze dynamic looping in association with MUC5B<br>• Define the contribution of HIF to molecular regulation of MUC5B. | 24-30 | Gerber, Colgan Evans | |
| *Milestone #1: Co-author manuscript on molecular regulation of MUC5B and variant* | | Colgan Gerber, Evans | |
| **Specific Aim 3:** ***Elucidate the impact of MUC5B variant on airway wound healing and proteostasis*** | | | |
| Subtask 1: ***Elucidate the influence of MUC5B variant on UPR and epithelial proteostasis utilizing airway cell lines.*** | 30-40 | Colgan Gerber, Evans | |

| | | | |
|---|---|---|---|
| Subtask 2: ***Define the impact of MUC5B rs35705950 variant on epithelial wound healing and barrier function.***<br>• Studies in cell lines, mouse models of airway fibrosis. | 40-48 | Colgan, Gerber Evans | |
| *Milestone #2: Co-author manuscript on functional impact of MUC5B variant in airway epithelia.* | | Colgan, Gerber, Evans | |

**Project 3**

**Site 1:** University of Colorado (UCo)
12700 East 19th Ave, MS 8611
Aurora, CO 80045
PI: Dr. Christopher Evans

**Site 2:** University of Alabama at Birmingham
1918 University Blvd
Birmingham, AL 35294
Co-I: Dr. Steven Rowe

| **Specific Aim 1: Demonstrate that MUC5B/Muc5b overproduction by club cells and type II cells in distal airways promotes dysfunctional MCC.** | **Timeline Years 1-4** | **Site 1** |
|---|---|---|
| **Major Task 1:** Regulatory approval, establishment of mouse colonies. | Months | |
| Subtask 1: Regulatory approval of animal research. | | Evans |
| *Milestone #1: Secure IACUC approval at University of Colorado.* | 0-3 | |
| *Milestone #2: Secure ACURO approval.* | 0-3 | |
| Subtask 2: Animal breeding for experiments. | | *All UCo investig.* |
| *Milestone #1: Import C57BL/6J mice and ROSA$^{mT/mG}$ strains.* | 0-3 | |
| *Milestone #2: Breed C57BL/6J, Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1Cre$^{ER/+}$;Muc5b$^{lox/lox}$; SftpcCre$^{ERT2/+}$;Muc5b$^{lox/lox}$; ROSA$^{mT/mG}$ mice.* | 3-18 | |
| **Major Task 2:** Demonstrate that Muc5b overproduction in murine PF impairs MCC and mucus transport *in vivo* and *in vitro*. | Months | |
| *Milestone #1: Acute and Chronic MCC in Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1-Muc5b$^{\Delta/\Delta}$; SftpcCre$^{ERT2/+}$;Muc5b$^{\Delta/\Delta}$* | 0-12 | Symmes, Hara |
| *Milestone #2: Mucus transport in primary lung epithelial cultures from Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1-Muc5b$^{\Delta/\Delta}$; SftpcCre$^{ERT2/+}$;Muc5b$^{\Delta/\Delta}$, & human cells ±IPF, ±rs35705950 'T'* | 0-12 | (7-10 mice/grp) (4-6 human culture/grp) |
| *Milestone #3: Statistical analysis of Data* | 12-18 | *All investig.* |
| **Major Task 3:** Demonstrate that aberrantly glycosylated MUC5B/Muc5b accumulates in the airways in PF. | Months | |
| *Milestone #1: Quantify MUC5B/Muc5b, SCGB1A1/Scgb1a1, SPC, MAL II, and UEA I labels human and mouse lung tissues by histology.* | 6-18 | Symmes, Hara (7-10 mice/grp) (8-12 human tissues/grp) |
| *Milestone #2: Demonstrate colocalization of secreted MUC5B/Muc5b with glycan markers* | 6-18 | |
| *Milestone #3: Statistical analysis of Data* | 12-18 | *All investig.* |
| *Milestone #4: Manuscript submission and publication: Aim 1 Data* | 9-18 | |
| **Major Task 4:** Determine whether an MCC defect in PrePF and IPF are associated with rs35705950 genotype. | Months | |
| *Milestone #1: Secure IRB approval at UAB for subject recruitment.* | 0-3 | Rowe, de Andrade |
| *Milestone #2: Perform rs35705950 genotyping* | 3-42 | Hara |
| *Milestone #3: Perform Tc99 MCC assays* | 3-42 | Rowe, de Andrade |
| *Milestone #4: Statistical analysis of Tc99 MCC data* | 3-48 | |
| *Milestone #5: Manuscript submission and publication: Human study.* | 42-48 | *All investig.* |

| | | |
|---|---|---|
| **Specific Aim 2: Determine whether Muc5b-dependent pro-fibrotic effects in mice are induced by aberrant mucin biosynthesis and proteostasis programs in club cells and T2 cells** | **Timeline Years 2-3** | |
| **Major Task 1:** Characterize the dependence of glycosylation of Muc5b on gene expression levels and cellular source. | Months | |
| *Milestone #1: Demonstrate colocalization of intracellular MUC5B/Muc5b with glycan markers and cell specific markers.* | 13-24 | Symmes, Hara (7-10 mice/grp) (8-12 human tissues/grp) |
| *Milestone #2: Identify changes in polymer size and migration by Western.* | 13-24 | |
| *Milestone #3: Statistical analysis of Data* | 16-27 | *All investig.* |
| **Major Task 2:** Determine the effects of Muc5b levels and localization on mucin biosynthetic enzyme expression in bleomycin-induced fibrosis. | Months | |
| *Milestone #1: Isolated and purify cells from fluorescent-tagged mice* | 20-26 | Symmes, Hara (7-10 mice/grp) (8-12 human tissues/grp) |
| *Milestone #2: Analyze St3Gal1- St3Gal6, St6Gal1- St6Gal2, Fut1-Fut11, and Agr2 transcript and protein levels.* | 24-27 | |
| *Milestone #3: Statistical analysis of Data* | 27-30 | *All investig.* |
| **Major Task 3:** Determine the effects of Muc5b levels and localization on proteostasis dysfunction in bleomycin-induced fibrosis. | Months | |
| *Milestone #1: Identify significant UPR/ER stress markers Atf6, Ern1(IRE-1α), Ern2 (IRE-1β), Ddit3 (CHOP), Hspa5 (Grp78/BiP), Eif2ak3 (PERK), and Xbp1/spliced Xbp1 in Muc5b-overexpressing mice.* | 24-27 | Symmes, Hara (7-10 mice/grp) (8-12 |
| *Milestone #2: Confirm protein levels & localization of markers above.* | 27-30 | human tissues/grp) |
| *Milestone #3: Statistical analysis of Data* | 30-32 | *All investig.* |
| **Major Task 4:** Effects of mucin biosynthesis and proteostasis regulators on Muc5b protein synthesis and pro-fibrotic mediator production. | Months | |
| *Milestone #1: Test ER Stress activation in MUC5B-expressing lung epithelial cell lines (A549, NCI-H292, and LC-2/ad) and NHBE's.* | 24-36 | Symmes, Hara (7-10 mice/grp) (8-12 human tissues/grp) |
| *Milestone #2: Test significance of ER Stress activation using lentiviral overexpression and shRNA-mediated knockdown.* | 30-36 | |
| *Milestone #3: Statistical analysis of Data* | 24-36 | *All investig.* |
| *Milestone #4: Manuscript submission and publication: Aim 1 Data* | 27-36 | |
| **Specific Aim 3: Determine the critical mechanisms required for MUC5B/Muc5b to promote pulmonary fibrosis.** | **Timeline Years 2-4** | |
| **Major Task 1:** In vivo studies. | Months | |
| *Milestone #1: Breed St3gal3, Fut2, Agr2, and Ern2 (IRE-1β) knockout mice for experiments. Obtain other candidates as needed.* | 13-44 | Symmes, Hara |
| *Milestone #2: Test effects genetic deficiency in* in vivo *models above on Muc5b levels, localization, and glycosylation and on epithelial proteostasis, ER stress, and fibrosis.* | 24-36 | Symmes, Hara (7-10 |

| | | |
|---|---|---|
| *Milestone #3: Test effects of pharmacologic and enzyme interventions in* in vivo *models above on Muc5b levels, localization, and glycosylation and on epithelial proteostasis, ER stress, and fibrosis.* | 33-44 | mice/grp) |
| **Major Task 2:** In vitro studies. | Months | |
| *Milestone #1: Test effects genetic deficiency in models above on mucus transport, and epithelial expression of pro-fibrotic mediators* in vitro. | 38-41 | Symmes, Hara (7-10 mice/grp) (4-6 human culture/grp) |
| *Milestone #2: Test effects of pharmacologic and enzyme interventions on mucus transport, and expression of pro-fibrotic mediators* in vitro. | 40-46 | |
| **Major Task 2:** Analysis and dissemination of Research. | Months | |
| *Milestone #1: Statistical analysis of Data* | 13-48 | *All investig.* |
| *Milestone #2: Manuscript submission and publication: Aim 1 Data* | 28-48 | |

**Projected Quarterly Enrollment**

| | **Year 1** | | | | **Year 2** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
| UAB | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Target Enrollment** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** |
| | **Year 3** | | | | **Year 4** | | | |
| | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
| UAB | 3 | 3 | 3 | 3 | 3 | 3 | 3 | - |
| **Target Enrollment** | **27** | **30** | **33** | **36** | **39** | **42** | **45** | **45** |

## **Project 4**

Site 1: University of Colorado Anschutz Medical Campus (AMC) 12700 East 19th Avenue, 8611, Aurora, CO 80045 Initiating PI: Dr. Ivana Yang

| **Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.** | **Timeline Years 1-2** | **Site 1** |
|---|---|---|
| **Major Task 1:** Regulatory approval and animal breeding. | Year 1 | |
| Subtask 1: Regulatory approval of animal research. | | |
| *Milestone #1: Secure IACUC approval at University of Colorado.* | month 0 | Dr. Yang |
| *Milestone #2: Secure ACURO approval.* | months 0-3 | |
| Subtask 2: Animal breeding for experiments. | | |
| *Milestone #2: Breed enough Muc5b-/-, Scgb1a1-Muc5b$^{Tg}$ and SPC-Muc5b$^{Tg}$ for experiments to commence.* | month 3-9 | Ms. Davidson |
| **Major Task 2:** Markers of ciliogenesis (Arl13b and Foxj1), Muc5b and Mmp7 will be co-localized with basal cell markers (Krt5, Krt14, and p63) and β-catenin following injury. | Years 1-2 | |
| *Milestone #1: Treat Muc5b-/-, Scgb1a1-Muc5b$^{Tg}$ and SPC-Muc5b$^{Tg}$ mice with bleomycin and H1N1 virus. Collect tissue for IF staining.* | months 3-12 | Ms. Davidson, Dr. Evans, Dr. Yang |
| *Milestone #2: Perform IF staining, take images, and perform qualitative analysis of the image data.* | months 12-18 | Fellow TBN, Ms. Davidson, Dr. Yang, Dr. Brody |
| *Milestone #3: Perform quantitative analysis of the image data and statistical analysis.* | months 18-24 | Fellow TBN, Drs. Eickelberg, Yang |

| | | |
|---|---|---|
| **Major Task 3:** Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury. | Years 1-2 | |
| *Milestone #1: Treat Muc5b-/-, Scgb1a1-Muc5b$^{Tg}$ and SPC-Muc5b$^{Tg}$ mice with bleomycin and H1N1 virus.* | months 3-9 | Ms. Davidson, Dr. Evans, Dr. Yang |
| *Milestone #2: Perform fresh lung tissue digests, DASC isolation, and RNA extractions.* | months 3-12 | Fellow TBN, Dr. Eickelberg, Dr. Yang, Dr. Mason |
| *Milestone #3: Run RT-qPCR on the Fluidigm platform.* | months 12-15 | Genomics Core, Dr. Bentley |
| *Milestone #4: Statistical analysis of RT-qPCR data and prioritization of genes for Aim 2.* | months 15-18 | Fellow TBN, Drs. Schwartz, Yang |
| **Major Task 4:** Publication of findings from Aim 1. | Year 2 | |
| *Milestone #1: Prepare and submit manuscript.* | months 18-24 | Site 1 personnel |
| **Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.** | **Timeline Years 1-4** | **Site 1** |
| **Major Task 1:** Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations. | Year 1-2 | |
| *Milestone #1: Establish NHBE cultures, successfully inhibit and overexpress positive control genes.* | months 0-9 | Fellow TBN, Dr. Königshoff, Dr. Yang, Dr. Chu |
| *Milestone #2. Optimize bleomycin and H1N1 virus concentrations.* | months 9-18 | |
| **Major Task 2:** Inhibit and overexpress cilium genes, measure injury/repair, regeneration, and Wnt signaling. | Years 2-3 | |
| *Milestone #1: Inhibit and overexpress cilium genes of interest.* | months 18-27 | Fellow TBN, Dr. Königshoff, Dr. Yang, Dr. Chu |
| *Milestone #2: Treat cells in which cilium genes are inhibited/overexpressed with bleomycin and H1N1.* | months 24-30 | |
| *Milestone #3. Measure wound healing, TEER, Wnt signaling.* | months 27-33 | |
| *Milestone #4: Statistical analysis of the data and prioritization of genes for Aim 3.* | months 30-36 | Fellow TBN, Drs. Königshoff ,Yang |
| **Major Task 3:** Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice. | Years 1-4 | |
| *Milestone #1: Breed Arl13$^{flox/flox}$ and Ift8$^{flox/flox}$ to Krt5-CreER mice. Breed CKO mice to Muc5b Tg or deficient lines. Treat with tamoxifen.* | months 3-18 | Ms Davidson, Dr. Evans, Dr. Yang |
| *Milestone #2: Treat mice with bleomycin and H1N1. Collect tissue for analysis.* | months 18-30 | Fellow TBN, Dr. Evans, Dr. Yang |
| *Milestone #3. IF staining for Arl13b, Foxj1, Muc5b, Mmp7 Krt5, Krt14, p63, and β-catenin.* | months 30-36 | Fellow TBN, Drs. Königshoff, Yang |
| *Milestone #4. Measure collagen content of the lung by hydroxyproline and SHG assays.* | months 30-36 | Fellow TBN, Dr. Evans, Dr. Yang |
| *Milestone #5: Statistical analysis of the data and prioritization of genes for Aim 3b.* | months 36-39 | Fellow TBN, Drs. Schwartz & Yang |
| **Major Task 4:** Publication of findings from Aim 2. | Year 4 | |
| *Milestone #1: Prepare and submit manuscript.* | months 36-42 | Site 1 personnel |

| **Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung** | **Timeline Years 1-4** | **Site 1** |
|---|---|---|
| **Major Task 1:** Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury. | Years 1-3 | |
| *Milestone #1: Perform IF staining, take images, and perform qualitative analysis of the image data in IPF and control lungs.* | months 0-18 | Fellow TBN, Dr. Eickelberg, Dr. Yang, Dr. Brody |
| *Milestone #2: Perform quantitative analysis of the image data and statistical analysis.* | months 12-36 | |
| **Major Task 2:** Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant. | Years 1-4 | |
| *Milestone #1: Perform fresh lung tissue digests, DASC isolation, and RNA extractions from IPF and control lungs.* | months 0-36 | Fellow TBN, Drs. Eickelberg, Yang |
| *Milestone #2: Run RT-qPCR Taqman assays for genes from Aims 1- 2.* | months 36-39 | Fellow TBN, Dr. Yang |
| *Milestone #3: Statistical analysis of RT-qPCR data.* | months 36-42 | Fellow TBN, Drs. Eickelberg, Yang |
| **Major Task 4:** Publication of findings from Aim 3. | Year 4 | |
| *Milestone #1: Prepare and submit manuscript.* | months 42-48 | Site 1 personnel |

## b. What was accomplished under these goals?

**Project 1**

- Approval from DoD and IRBs at University of Colorado, National Jewish (cede to University of Colorado), University of Alabama Birmingham, and University of Pittsburgh to begin study
- Recruitment of research participants referred from University of Colorado, National Jewish Health, University of Alabama Birmingham, and University of Pittsburgh
- Approval from University of California San Francisco and Vanderbilt University/Colorado Multiple Institutional Review Board (COMIRB) – pending DoD approval to begin enrollment
- Pending approval from COMIRB and the VA for study initiation
- Recruitment of human participants is ongoing at open sites
- 105 first degree relatives of people with IPF referred for study participation
- 64 first degree relatives of people with IPF consented to study participation
- 36 first degree relatives of people with IPF have completed some, but not all study procedures
- 28 first degree relatives of people with IPF have completed all study procedures (informed consent, health questionnaire, blood draw, HRCT scan)
- Radiologic and clinical evaluation by thoracic radiologists and interstitial lung disease specialist clinicians of completed subjects is in process and ongoing
- No adverse events in the human subjects study

Significant Results: In 496 familial interstitial pneumonia (FIP) relatives from 263 distinct FIP families, the prevalence of pre-clinical pulmonary fibrosis (PrePF) on visual CT evaluation was 15·5%. The deep-learning quantitative CT method had 74% sensitivity and 82% sensitivity for detecting PrePF. PrePF subjects were older (65.8, 95% CI 63.5-68.1 years) than subjects without fibrosis (55·8, 95% CI 54·9-56·6 years, p=6·36 x10-$^{13}$), more likely to be male (48% versus 36%, p=0·05), more likely to have smoked (43% versus 27%, p=0.007), and to have the *MUC5B* promoter variant rs35705950 (minor allele frequency 0·29 versus 0·21, p=0·025). *MUC5B* variant carriers had higher quantitative CT fibrosis scores (1·3 [95% CI 1·2-1·5] versus 1·1 [95% CI 1·0-1·2, p=0·03] controlling for age and sex. PrePF is prevalent in FIP relatives. Its prevalence increases with age and with the presence of a common *MUC5B* promoter variant. Quantitative CT can detect this early form of fibrosis.

In addition, we found that circulating plasma proteins that are differentially detected in IPF subjects are also differentially detected in subjects with PrePF. These protein levels could be useful in identifying individuals with early forms of pulmonary fibrosis or at highest risk of developing disease.

**Project 2**

Major activities

1. Performed ChIP assays on MUC5B enhancer region and demonstrated that region is hyperchippable, and has the capacity to interact with many proteins including HIF1 (Aim1, subtask 1)
2. Performed analysis of chromatin structure of regions around SNP using MNase assays and histone chip (Aim 1, subtask 1)
3. Tested HIF-1 binding to the MUC5B promoter by chromatin immunoprecipitation (Aim 1, Subtask 1)
4. Generated and tested edited clones of A549 cells (Aim1, Subtask 2)
5. Presented poster and published abstract on MUC5B regulation at ATS international conference in 2018 (Aim 1, Milestone 1)
6. Tested human GCF, HIF-1 and HIF-2 knock-down cell lines and the contribution of GCF/HIF to MUC5B regulation (Aim 1, Subtask 3)

Significant Results: In the experiment shown below (Fig. 1), we used a high resolution micrococcal nuclease access assay to probe the chromatin structure of the MUC5B 5' -3kB enhancer in wild type A549 cells and CRISPR edited cells in which the G has been replaced with a T. These data

show two important findings. First there is a minimal or no effect of the variant on underlying chromatin structure. Second, the chromatin structure of this region has several areas that are highly accessible to micrococcal nuclease indicative of likely access to transcription factors, providing a potential underlying mechanism for the ”leaky” aberrant expression of MUC5B that it's observed in idiopathic pulmonary fibrosis.

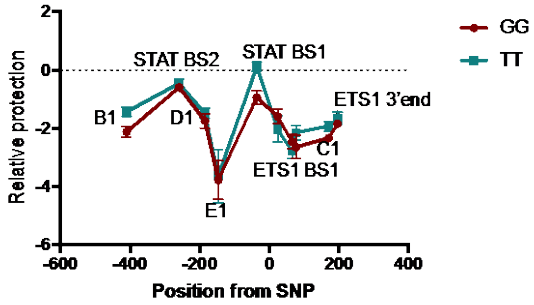



**Figure 1: MNase Access assay**

In Figure 2, experiments showing that the transcription factor SPDEF regulates MUC5B expression through a binding site adjacent to the variant region are shown. In particular, luciferase assays with and without transfection of an SPDEF expression construct in wild type and constructs with mutations in a putative SPDEF (ETS-family) binding site are shown. Note the inductive effect of SPDEF that is abrogated with mutation of the binding site. The left side is a short version of the -3kB enhancer, the right side is a full length version.

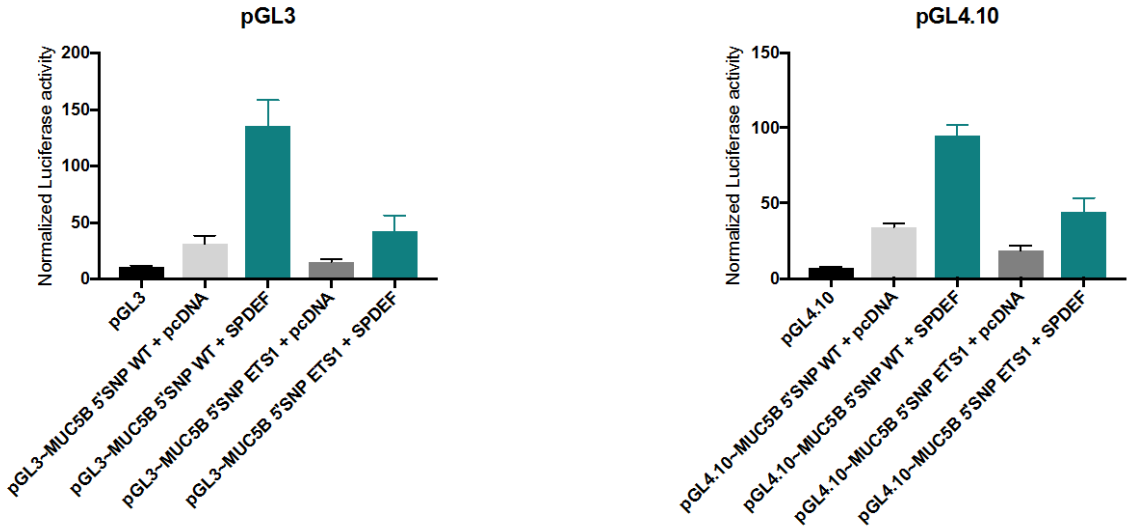



**Figure 2. SPDEF regulates the MUC5B -3kB enhancer through a conserved ETS-family binding site.**

As proposed in Specific Aim 1 and as part of our analysis this past year, we have profiled the impact hypoxia and HIF on the expression and regulation of the Muc5B promoter. As shown in Figure 3 (left panel), we examined how pharmacological stabilization of HIF using IOX-2 might impact Muc5B promoter activity in A549 cells. As can be seen, IOX-2 significantly elevated Muc5B promoter activity that was attenuated in the HIF-mutant promoter construct. These results provide insight into regulatory pathways that we can now pursue at the molecular level.

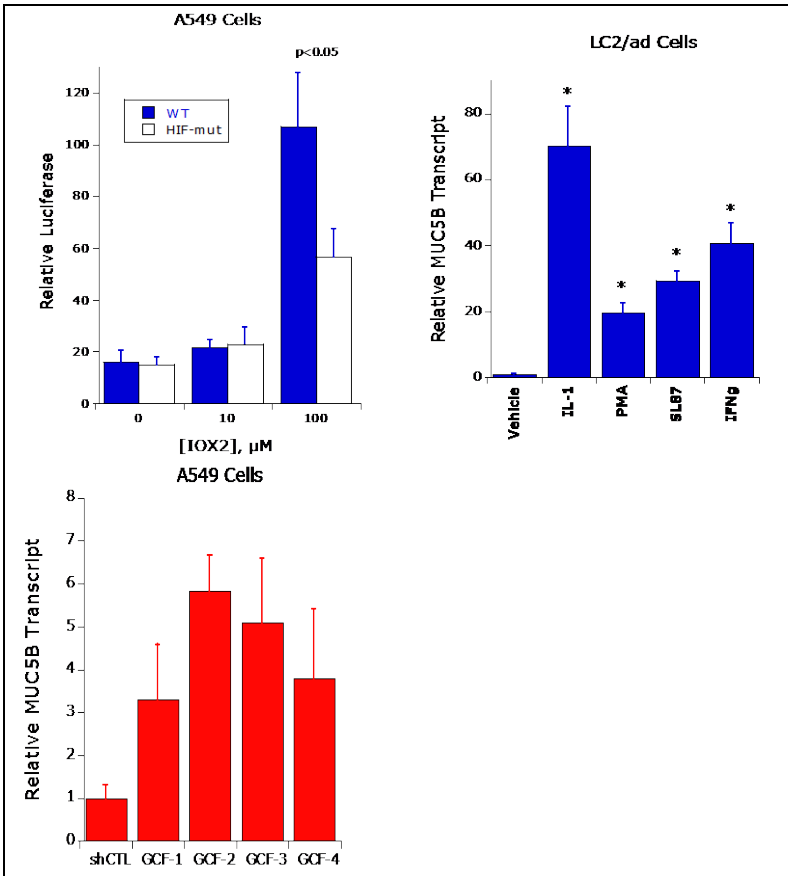



**Figure 3. Regulation of Muc5B by pharmacological HIF stabilization in A549 cells (left), by inflammatory stimuli in LC2/ad cells (middle panel) and by lentiviral-mediated knockdown of the transcription factor GCF (right panel). See text for details.**

Likewise, as proposed in Specific Aim 1, we examined how different inflammatory stimuli regulate Muc5B in LC2/ad cells (Figure 3, middle panel). These results indicate that the cytokines IL-1-beta and IFN-gamma prominently induce Muc5B mRNA in LC2/ad cells. Similarly, the phorbol ester PMA and the HIF stabilizer SL87 induce Muc5B. Such results provide important guidance for targeted regulatory examination of the Muc5B promoter.

We also examined the impact of the transcription factor GCF on expression of Muc5B (Figure 3, right panel), as proposed in Aim 1. To do this, we targeted knockdown of GCF using 4 different lentiviral constructs and compared stabled transduced cells to short-hairpin controls (shCTL). These studies revealed that knockdown of GCF significantly induces Muc5B transcript, validating our original preliminary data that GCF is a transcriptional repressor of Muc5B.

**Project 3**
**Specific Aim 1: Demonstrate that MUC5B/Muc5b overproduction by club cells and T2 cells in distal airways promotes dysfunctional MCC.**

**Major Task 1:** Regulatory approval, establishment of mouse colonies

- All regulatory work was completed, and mouse colonies are established and breeding.
- Mice are breeding and have been assigned to or used in studies of acute and chronic MCC.

**Major Task 2:** Demonstrate that Muc5b overproduction in murine PF impairs MCC and mucus transport in vivo and in vitro.

- We developed and refined strategies for clean isolation and assessment of lung bacteria (chronic MCC), have established additional imaging modalities, and begun culturing airway epithelial cells.
- One line, *St3gal3* knockouts, has bred poorly. We are adding more animals at a 50% slower than expected rate.
- We are only part way through human cell analyses. We are obtaining greater numbers of TT cells and working through culturing of these.

**Major Task 3:** Demonstrate that aberrantly glycosylated MUC5B/Muc5b accumulates in the airways in PF.

- Milestone #1: Quantify MUC5B/Muc5b, SCGB1A1/Scgb1a1, SPC, MAL II, and UEA I labels human and mouse lung tissues by histology.
- Milestone #2: Demonstrate colocalization of secreted MUC5B/Muc5b with glycan markers
- Studies are underway presently, and as of 10/1/18 include 7 IPF GG and 5 IPF TT patient samples.
- Four manuscripts published or in press.
    - Nature Communications, in press
    - Annals ATS, in press
    - Biochem Soc Trans. 2018 Jun 19;46(3):707-719. doi: 10.1042/BST20170455.
    - Am J Respir Cell Mol Biol. 2018 Sep;59(3):287-288. doi: 10.1165/rcmb.2018-0141ED.

**Major Task 4:** Determine whether an MCC defect in PrePF and IPF are associated with rs35705950 genotype.

- Milestone #1: Received DOD approval to enroll, with minor adjustments of our previously UAB IRB approved protocol (essentially adjusting funding source clarifications in the informed consent form). Research Coordinator presence was increased in the clinic to support increased enrollment. Patients in the UAB population were approached about enrolling in multiple studies at clinic visits. This became a potential barrier to recruitment, so the Research Coordinator and the Clinic Coordinator identified an approach to increase enrollment and also ensure patients are not overwhelmed with research requests at clinic visits.

  By the end of Y1, UAB screened N=10 patients, with a total enrollment of N= 9. In addition, over 40 have been pre-screened for eligibility and declined participation. N=1 participant scheduled for mid-October. Note N=4 patients were enrolled prior to the inception of funding and DOD approval, but will be included in the final analysis since this was performed under UAB IRB approval with institutional support.

**Specific Aim 2: Determine whether Muc5b-dependent pro-fibrotic effects in mice are induced by aberrant mucin biosynthesis and proteostasis programs in club cells and T2 cells.**

**Major Task 1:** Characterize the dependence of glycosylation of Muc5b on gene expression levels and cellular source.

- We have developed and assessed the amounts and glycosylation of Muc5b in mouse models at baseline
- We identified changes in polymer size and migration by Western after inflammatory challenge and are deploying this in mice at different times post bleomycin

**Major Task 2:** Determine the effects of Muc5b levels and localization on mucin biosynthetic enzyme expression in bleomycin-induced fibrosis.

- We developed novel isolation and purification methods for lung epithelia
- We developed assays for screening St3Gal1- St3Gal6, St6Gal1- St6Gal2, Fut1-Fut11, and Agr2 transcript and protein levels.

**Major Task 3**: Determine the effects of Muc5b levels and localization on proteostasis dysfunction in bleomycin-induced fibrosis.

- We developed assays for Atf6, Ern1(IRE-1α), Ern2 (IRE-1β), Ddit3 (CHOP), Hspa5 (Grp78/BiP), Eif2ak3 (PERK), and Xbp1/spliced Xbp1

**Specific Aim 3: Determine the critical mechanisms required for MUC5B/Muc5b to promote pulmonary fibrosis.**

**Major Task 1:** In vivo studies.

- We obtained Ern2 (IRE-1β) knockout mice from an outside source.
- We are breeding St3gal3, Fut2, and Ern2 knockout mice for experiments.

**Project 4**

**Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.**

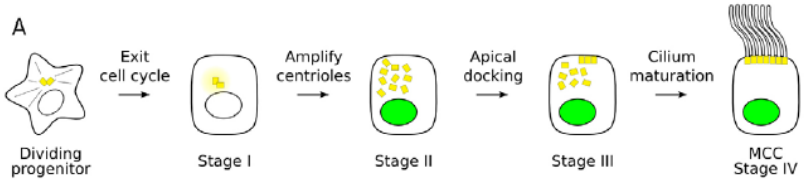
Major Task 1: Regulatory approval and animal breeding (scheduled for months 0-9; 100% complete). We obtained IACUC approval at the University of Colorado 06-09-2017 and ACURO approval 09-05-2017 prior to start of funding and therefore ahead of the proposed milestone. Muc5b strain breeding commenced immediately after funding started and we have been able to breed sufficient numbers of animals to stay on track with experiments proposed in Aim 1. This task is completed.

Major Task 2: Markers of ciliogenesis (Arl13b and Foxj1), Muc5b and Mmp7 will be co-localized with basal cell markers (Krt5, Krt14, and p63) and β-catenin following injury (scheduled for months 3-24; 50% complete).

We have collected tissue from Scgb1a1-Muc5bTg, SPC-Muc5bTg, wild type, and Muc5b-deficient mice using two bleomycin models:

1. Single i.t. dose: 2.5U/kg of bleomycin or saline on week 0; tissue collection at 1, 2, 3, 7, and 10 weeks
2. Repeat i.t. dose: 2.5 U/kg on week 0, 1.5 U/kg week 2 and week 4; tissue collection week 7 and 10

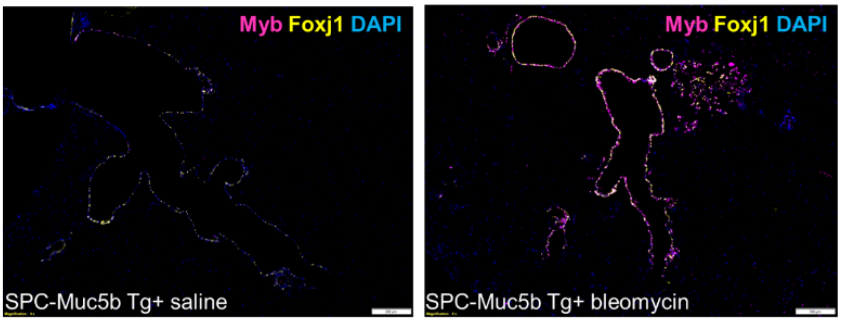
We have also collected tissue from Scgb1a1-Muc5bTg, SPC-Muc5bTg, wild type, and Muc5b-deficient mice treated with H1N1/Puerto Rico/8/34 virus (ATCC VR-95) or saline i.n. for 1, 2, 3, or 16-20 weeks.

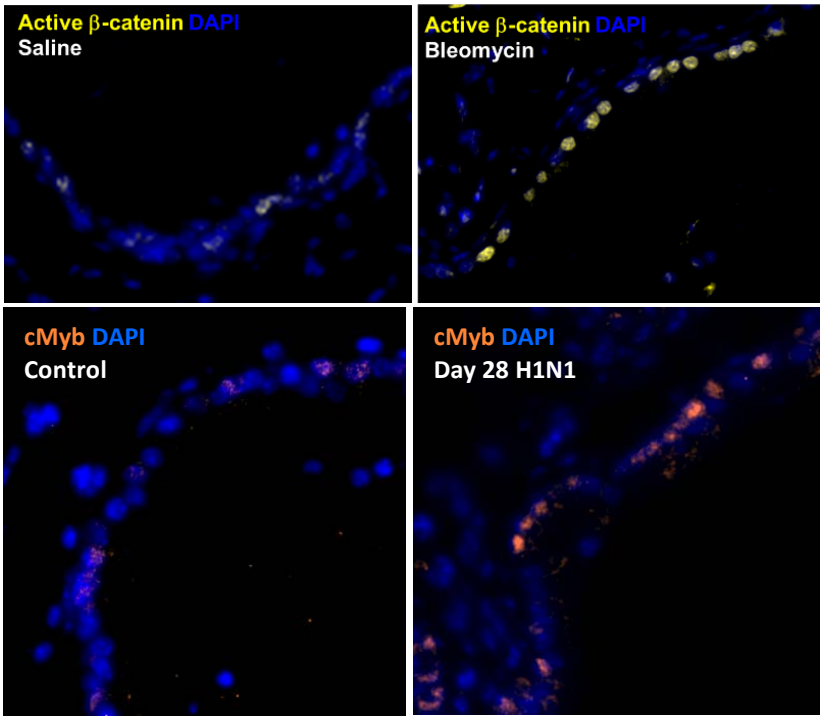



| Immunofluorescence (IF) | Stage of ciliogenesis | Primary/ motile cilia |
|---|---|---|
| Krt5$^+$; Arl13b$^+$; Myb$^-$; Foxj1$^-$ | Primary cilia | 1~2 mm of primary cilia |
| Krt5$^+$; Arl13b$^+$; Myb$^+$; Foxj1$^-$ | Stage I | 5~10 mm of primary cilia |
| Krt5$^+$; Arl13b$^-$; Myb$^+$; Foxj1$^+$ | Stage II | Disappear |
| Krt5$^+$; Arl13b$^-$; Myb$^-$; Foxj1$^+$ | Stage III | Axonome do not appear prior to docking |
| Krt5$^+$; Arl13b$^+$; Myb$^-$; Foxj1$^+$ | Stage IV | 1~5 mm of motile axoneme |

*Note: IF positive no ciliogenesis*

We have implemented and tested an immunofluorescence panel of antibodies that allows us to determine the stage of ciliogenesis in KRT5 positive cells (Tan. Development 2013;140:4277-4286). We have also implemented a panel for nuclear localization of β-catenin in KRT5 positive cells. The results of the first set of qualitative analysis of IF staining demonstrate active multiciliogenesis, as evidenced by increased Myb staining, occurring at week 10 following bleomycin injury in both single and repeat models (10 weeks after the last dose of bleomycin in the repeat model), especially in Muc5b Tg overexpressing strains of mice. This timepoint corresponds to the repair process following bleomycin injury. We are in the process of determining peak of ciliogenesis in the H1N1 model. The first set of IF staining demonstrated that increased Myb staining found at 28 days post H1N1 exposure. We also started collecting data on nuclear β-catenin in these models. This task is on track to be completed on time.




Major Task 3: Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury (scheduled for months 3-18; 25% complete).

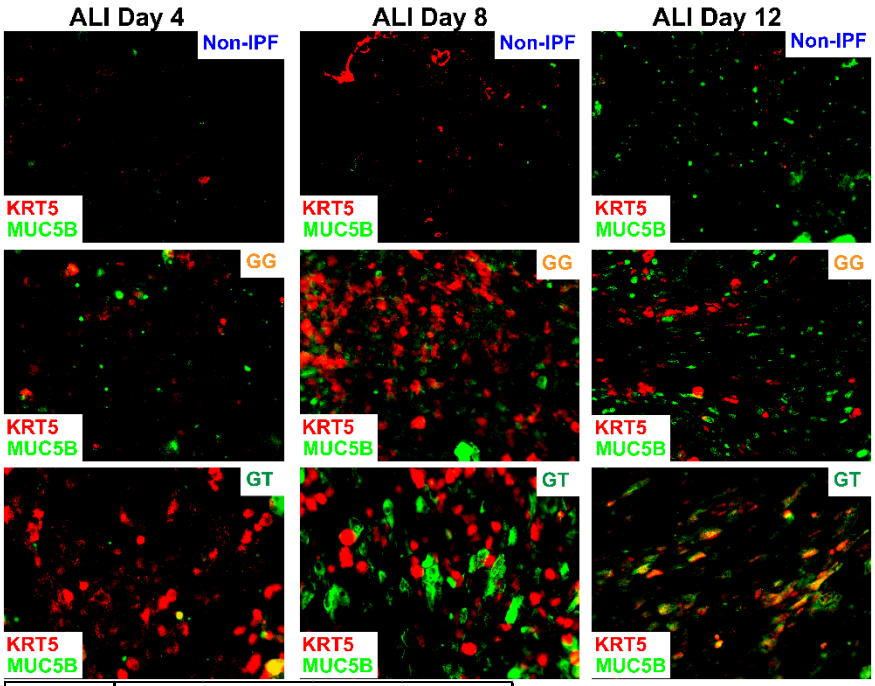
We have successfully isolated and characterized CD326(EPCAM)+ cell populations from Muc5b Tg- and Tg+ mice. Dispase digestion methods were used to isolate lung cells followed by removal of CD45 and CD31 cells by magnetic beads. Isolated cells were labeled with antibodies to CD326 and fixed with 1% paraformaldehyde. Only CD326 positive cells were sorted and used to characterize the major cell population. 1 million epithelial cells were collected per mouse. We have characterized the major cell populations present at baseline (prior to treatment with bleomycin) and these results show that 1% of the cells are Krt5+ (this percentage is higher following injury). Given the small numbers and difficulty of isolating pure Krt5 cell populations, we are in the process of breeding enough mice to isolate Krt5 positive cells using the mTmG tag in Krt5-CreERT2-

RosamT/mG; upon treatment with tamoxifen, Krt5 cells will be labeled with GFP and we can use flow cytometry to sort them using GFP. Mice will be ready by December and sample collection will be done by February 2019. This task is slightly delayed due to the difficulties we have encountered with cell isolations but we should be able to get back on track in the upcoming year.
We will also characterized the profile of other lung cells types (Type II cells, ciliated cells, club cells) to understand the disease progression over time following injury. We will also use ImageStream, imaging cytometer, to precisely determine small population of epithelial cells.

**Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.**

Major Task 1: Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations (scheduled for months 0-18; 50% complete).

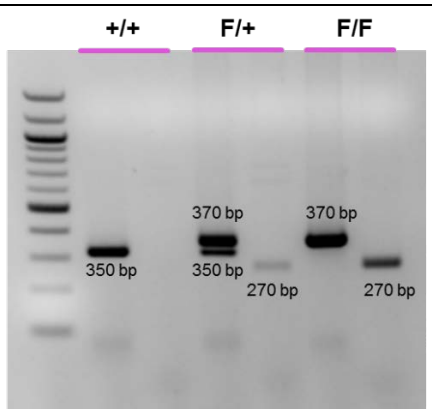
We have begun culturing NHBE cells, have optimized bleomycin concentration (to observe an increase in MUC5B protein secretion with no cell death; FC=fold change in the table below), and are in the process of optimizing H1N1 concentration. We have not yet performed lenti-shRNA and lenti-ORF protocols on control genes but will begin that work this year. This task is on track to be completed on time.

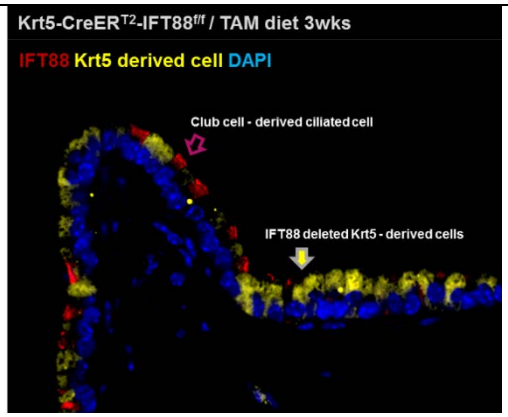



| | secreted MUC5B protein | | | |
|---|---|---|---|---|
| | 24hr FC | 24hr p val | 72hr FC | 72hr p val |
| **untreated** | 1.34 | 0.3109 | 1.73 | 0.1771 |
| **Bleo** | 1.73 | 0.2072 | 3.53 | 0.0439 |
| **Pam3Cys** | 2.03 | 0.1635 | 1.91 | 0.0721 |
| **poly(I:C)** | 2.05 | 0.0521 | 3.72 | 0.0507 |
| **LPS** | 2.89 | 0.1880 | 2.23 | 0.0776 |
| **TGFb** | 2.45 | 0.0560 | 2.52 | 0.1514 |

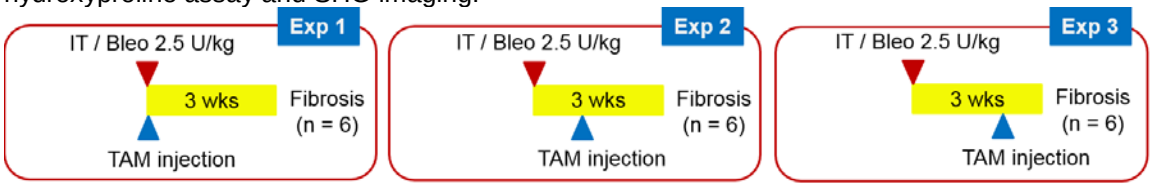
Major Task 3: Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice (scheduled for months 3-39; 12.5% complete).

At the beginning of this funding period, we imported Ift88 flox/flox and Krt5-CreER mice, and begin to breed them to generate Krt5-Ift88 flox/flox mice. Tamoxifen diet resulted in inefficient Cre recombinase activity and we switched to tamoxifen i.p. injections in corn oil. i.p. injection of 100 mg/kg tamoxifen for 4 days results in sufficient recombination. Immunofluorescence demonstrates presence of Club cell-derived ciliated cells, suggesting that we may need to consider breeding to Ift88 flox/flox and Scgb1a1-CreER mice if the results of the Krt5-Ift88 flox/flox are negative.




We currently have three experimental groups to test the effect of Ift88 deletion on development of fibrosis. As outlined in the original proposal, we will measure collagen content assessed by hydroxyproline assay and SHG imaging.

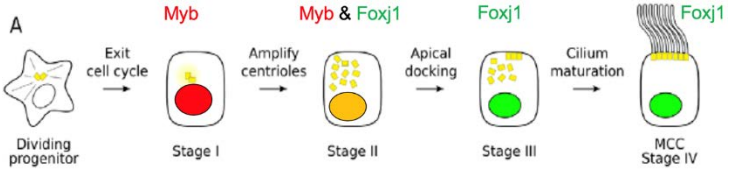


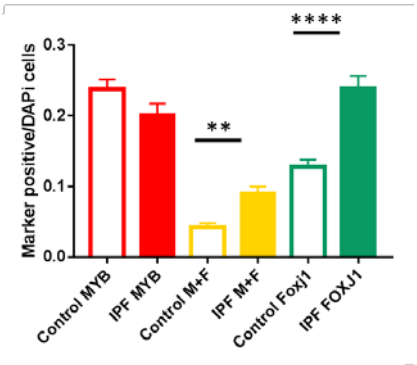

Arl13b flox/flox breeder mice were imported from Emory University and Mice will be ready by the end of October. This task is on track to be completed on time.

**Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung.**

Major Task 1: Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury (scheduled for months 0-36; 25% complete).

Using the same panel of markers for ciliogenesis as in Aim 1, we have begun to quantify the extent of ciliogenesis in small airways of IPF and control subjects. Results from the first 8 subjects (2 GT IPF, 2 GG IPF, 2 GT control, and 2 GG control) show more active ciliogenesis in IPF compared to controls. We continue to stain and quantify these markers in additional subjects. This task is on track to be completed on time.




Major Task 2: Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant (scheduled for months 0-42; 5% complete).

We have collected cells from 2 IPF and 2 control subjects so far. This task is on track to be completed on time.

**Biostatistics Core**

During the first year we have completed the hiring process for both a biostatistician and computer programmer who are supported part-time on this award. Both have played a key role in the

analysis if our genetic data that is key for identification of the variants we will use in our models with the first degree relatives. Specifically, Mr. Jacobson has become familiar with all of the IPF GWAS data and learned the process for imputation to 1000 Genomes of our data, helping complete those analyses. In addition, he has been trained on the methods used to calculate and display statistics related to the predictive properties of single variants or combinations, including ROC curves, negative predictive value, positive predictive value, sensitivity and specificity. Mr. Anderson has developed the visualization tools for each of these items in addition to developing a general tracking system for release of data sets to investigators in each project if/when those are prepared later in the award. Dr. Fingerlin is available to all project investigators as it relates to study design or other questions; since this was the first year of the award, few changes have been made that require redesign of planned analyses, etc.

**Administrative Core**
During the first year, we executed and monitored subcontracts for all six sub-sites (University of California San Francisco, DRI/VA, University of Pittsburgh, Vanderbilt University, University of Alabama, and National Jewish Health. The Internal Advisory Committee (IAC) met two times during this project period and we are in the process of organizing a meeting for the External Advisory Committee (EAC).

## c. What opportunities for training and professional development has the project provided?

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

## d. How were the results disseminated to communities of interest?

**Project 1**
Manuscript under review at *Thorax* and abstract submitted to 2019 American Thoracic Society meeting.

**Project 2** – nothing to report

**Project 3**
Findings were presented at the 2018 American Thoracic Society Conference and in four publications.

- Nature Communications, in press
- Annals ATS, in press
- Biochem Soc Trans. 2018 Jun 19;46(3):707-719. doi: 10.1042/BST20170455.
- Am J Respir Cell Mol Biol. 2018 Sep;59(3):287-288. doi: 10.1165/rcmb.2018-0141ED.

**Project 4**
Dr. Yang presented results of analyses of ciliogenesis markers in the bleomycin mouse model and in IPF lung tissue at the FASEB The Lung Epithelium in Health and Disease Conference held July 29 – Aug 3, 2018. Dr. Eunjoo Kim presented her progress in the Lung and Regeneration Working Group meeting on October 12, 2018. Dr. Kim is submitting an abstract to present at the Gordon Cilia, Mucus and Mucociliary Interactions Research Conference to be held on Feb 17-22, 2019.

**Biostatistics Core –** nothing to report

## e. What do you plan to do during the next reporting period to accomplish the goals?

**Project 1**

- Referral, recruitment, and enrollment of research participants will continue in the next reporting period
- We anticipate approval from Vanderbilt, University of California San Francisco, and the Veterans Administration during the next reporting period and will begin referral and recruitment from these sites following approval
- Phenotyping of participants will continue in the next reporting period

**Project 2**

During the next reporting period, we will complete Aim 1 and begin the process of preparing and submitting a manuscript that reports on the role of chromatin structure in regulating MUC5B expression in relationship to the variant (Milestone 1). We will also ramp up our efforts on Aim 2, focusing on investigating the three-dimensional chromatin organization of the MUC5B locus. This work will include analysis of several enhancer regions that we have identified previously, and will determine whether these enhancers cooperate with the variant containing region to regulate MUC5B expression. We will also continue to perform knockdown and overexpression analysis using lentiviral constructs (e.g. GCF, HIF-1) to further delineate specific factors that regulate MUC5B expression and will utilize MUC5B CRISPR edited "TT" lines in comparison to "GG" lines to determine the impact of the variant on gene regulation. In addition, we will pursue mechanisms of Muc5B regulation by inflammatory mediators and HIF stabilizers (see Figure 3 of progress report).

**Project 3**

We will continue on Aim 1, Major Tasks 2, 3 and 4. To accomplish this we are performing mucociliary transport studies in all mouse lines and in additional human cells. We are also preparing an additional manuscript on transport function and mucus gel structure. UAB will continue enrollment, conduct of MCC scans, and collection of blood samples for genetic analysis. We are also continuing Aim 2 using mouse and human samples to determine mucin glycosylation and how this affects mucus gel structure, and mucous cell biosynthetic component levels.

For Aim 3, we are breeding mice onto appropriate backgrounds an assigning them to challenge groups.

**Project 4**

**Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.**

Major Task 2: Markers of ciliogenesis (Arl13b and Foxj1), Muc5b and Mmp7 will be co-localized with basal cell markers (Krt5, Krt14, and p63) and β-catenin following injury (scheduled for months 3-24; 50% complete).

We will complete all animal work in the H1N1 model, finalize immunofluorescence staining of ciliogenesis and quantify the results.

Major Task 3: Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury (scheduled for months 3-18; 25% complete).

We will isolate Krt5+ cells after injury with bleomycin and H1N1 and perform gene expression analysis.

Major Task 4: Publication of findings from Aim 1 (scheduled for months 18-24; 0% complete)

We plan on submitting the publication that describes the results from Aim 1 by the end of the next funding period.

**Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.**

Major Task 1: Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations (scheduled for months 0-18; 50% complete).
We will finish optimization of lenti-shRNA and lenti-ORF protocols by performing knockdown and overexpression of control genes.

Major Task 3: Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice (scheduled for months 3-39; 12.5% complete).
We will finish characterization of the model of deletion of Ift88 in Krt5-derived cells. If the results are promising, we will breed these mice to Muc5b Tg lines. If not, we will initiate the model of deletion of Ift88 in Scgb1a1-derived cells. We will also initiate the model of deletion of Arl13b in Krt5-derived cells.

**Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung.**
Major Task 1: Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury (scheduled for months 0-36; 25% complete).
We will continue to stain and quantify ciliogenesis in additional IPF and control subjects, and begin analysis by MUC5B genotype.
Major Task 2: Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant (scheduled for months 0-42; 5% complete).
We will continue to collect cells from IPF and control lung tissue.

**Biostatistics Core –** nothing to report

# 4. Impact

## a. What was the impact on the development of the principal discipline(s) of the project?

**Project 1**

- This study's enrollment of healthy relatives of people with sporadic IPF is spreading the idea that IPF can run in families even when there is no known family history, which is important for explaining risk of IPF. As we continue to reach out to physicians about patient recruitment, we share the knowledge that genetics are a key risk factor for IPF.

**Project 2**
Our results broaden the understanding of epigenetic regulation with respect to idiopathic pulmonary fibrosis, as we are focusing on aspects of chromatin structure that are not typically assayed in determining epigenetic regulatory mechanisms.

**Project 3**
We have championed a concept that is driving the pulmonary fibrosis field in a new direction. Along with other Program Project Grant teams, we are demonstrating that mucociliary dysfunction is an important and treatable phenomenon in lung fibrosis.

**Project 4**
Our work over the past year has identified critical timepoints at which ciliogenesis is active following lung injury, in the context of overproduction of the airway mucin MUC5B.

**Biostatistics Core –** nothing to report

### b. What was the impact on other disciplines?

**Project 1** – nothing to report

**Project 2**
The optimization of the MNASE qPCR assay to examine chromatin structure in airway cells can be applied to other airway cell types to examine epigenetic alterations in exposure-related lung disease, including military-relevant exposures such as particulate matter and ozone. Likewise, our results implicate an important role for the tissue microenvironment (e.g. tissue hypoxia, tissue inflammation) on the direct regulation of Muc5B.

**Project 3**
Our findings are carrying a concept of mucous cell proteostasis that is being tested in acute lung injury and asthma.

**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### c. What was the impact on technology transfer?

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### d. What was the impact on society beyond science and technology?

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

# 5. Changes/Problems

### a. Changes in approach and reasons for change

**Project 1**
No significant changes are anticipated. We will continue to emphasize physician and patient outreach to improve recruitment into the study.

**Project 2**
No significant changes are anticipated. Our discovery that the MUC5B enhancer is hyperchippable has minor implications for appropriate controls to use in ChIP assays, but will not substantially impact our proposed experiments.

**Project 3**
St3gal3 knockout mice breed poorly, and have pleiotropic abnormalities due to widespread expression of the gene. We are generating a conditional knockout line. Ern2 knockout mice were

sent to us on a 129Sv mouse strain background that is not ideal for pulmonary fibrosis modeling. We generated a C57BL/6 line using CRISPR/Cas9 targeting.

**Project 4**

1. We are using Myb and Foxj1, instead of Arl13b and Foxj1, as the markers of ciliogenesis that will be quantified because they are both localized to the nucleus and are best markers for multiciliogenesis.
2. We are using the mTmG tag in Krt5-CreERT2-RosamT/mG to isolate Krt5 cells for gene expression profiling; upon treatment with tamoxifen, Krt5 cells will be labeled with GFP and we can use flow cytometry to sort them using GFP. Out original plan was to use negative selection but that plan did not result in isolation of sufficient purity of Krt5 cells.

**Biostatistics Core –** nothing to report

## b. Actual or anticipated problems or delays and actions or plans to resolve them

**Project 1**

- Recruitment has been slower than expected. We are working to improve this in four ways:
  1) We continue to move forward with getting IRB approvals finalized from Vanderbilt, University of California San Francisco, and the VA so that we can maximize recruitment
  2) The Project 1 PI, Dr. David Schwartz, is increasing his 1-on-1 contact with site PIs to improve the pace of referrals for study participation from slower sites
  3) We are in the process of placing a study coordinator on site at NJH in the clinic where in the past all referrals had been by phone and email. This should improve the pace of referrals from NJH
  4) We are building a public website to share information about pulmonary fibrosis and also to assist in recruitment for this study

**Project 2** – nothing to report
**Project 3** – see above

**Project 4**

The main delay we experienced was in cell isolations due to difficulties we encountered. We now have a plan to use the mTmG tag in Krt5-CreERT2-RosamT/mG to isolate Krt5 cells.

**Biostatistics Core –** nothing to report

## c. Changes that had a significant impact on expenditures

**Project 1** – nothing to report
**Project 2** – nothing to report

**Project 3**

Knockout mice have cost approximately $5,000 to generate. This was relatively minor in terms of financial impact, but since it could cause delays in years 2-3, we started in year 1.

**Biostatistics Core –** nothing to report

## d. Significant changes in the use or care of human subjects, vertebrate animals, biohazards, and/or select agents

**Project 1** – nothing to report

**Project 2** – nothing to report
**Project 3** – nothing to report

**Project 4**
Postdoctoral Fellows Dr. Hui and Dr. Kim were not hired until early 2018 and this delayed some of the experiments.

**Biostatistics Core –** nothing to report

### e. Significant changes in use or care of human subjects

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### f. Significant changes in use or care of vertebrate animals

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### g. Significant changes in use of biohazards and/or select agents

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

## 6. Products

### a. Publications, conference papers, and presentations

**Project 1** – nothing to report

**Project 2**
Abstract presentation: ATS International Conference, May 2018, San Diego, CA
Transcriptional Regulation of MUC5B and the Enhancer Harboring the IPF-Associated Variant rs35705950
A.N. Gerber, F. Gally, S.K. Sasse, B.A. Helling, C.M. Evans, I.V. Yang, S.P. Colgan, and D.A, Schwartz

**Project 3**
Journal articles
Role of mucins in lung homeostasis: regulated expression and biosynthesis in health and disease. Symmes BA, Symmes AL, Magin CM, Evans CM. *Biochem Soc Trans*. 2018 Jun 19;46(3):707-719. doi: 10.1042/BST20170455. Epub 2018 May 25. PMID: 29802217

SPDEFending the Lung through Mucin Expression. Evans CM, Seibold MA, Gerber AN. *Am J Respir Cell Mol Biol.* 2018 Sep;59(3):287-288. doi: 10.1165/rcmb.2018-0141ED. PMID: 29723043

**Project 4**
Dr. Yang presented results of analyses of ciliogenesis markers in the bleomycin mouse model and in IPF lung tissue at the FASEB The Lung Epithelium in Health and Disease Conference held July 29-Aug 3, 2018. Dr. Kim is submitting an abstract to presented at the Gordon Cilia, Mucus and Mucociliary Interactions Research Conference to be held on Feb 17-22, 2019.

**Biostatistics Core –** nothing to report

### b. Website(s) or other Internet site(s)

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### c. Technologies or techniques

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### d. Inventions, patent applications, and/or licenses

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

### e. Other products

**Project 1** – nothing to report
**Project 2** – nothing to report
**Project 3** – nothing to report
**Project 4** – nothing to report
**Biostatistics Core –** nothing to report

# 7. Participants & Other Collaborating Organizations

**Project 1**

| | |
|---|---|
| **Name:** | **David Schwartz, MD** |
| **Project Role:** | Project Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |

| | |
|---|---|
| **Contribution to project:** | Responsible for the design and execution of the study, and the day-to-day functioning, trouble-shooting, integration, training, and long-term planning of the study. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Joyce Lee, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Lead the recruitment efforts for Aims 1 and 3. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Jill Norris, PhD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Longitudinally assess genetically at-risk cohorts for the appearance of autoantibodies and for the subsequent progression to clinical disease. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Marvin Schwarz, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Provide oversight for the clinical phenotyping of the subjects in this project. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Tasha Fingerlin** |
| **Project Role:** | Biostatistician |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Oversees all of the statistical analyses related to the biomarker discovery and validation work in relationship to Project 1. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Corinne Hennessy** |
| **Project Role:** | Professional Research Assistant |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 6 |
| **Contribution to project:** | Responsible for organizing, tracking, and curating the DNA and biological samples for this project, and the |

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|---|---|
| | follow up genotyping efforts and biomarker assays (mRNA and protein). |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Alex Jones** |
| **Project Role:** | Study Coordinator, UCD |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 12 |
| **Contribution to project:** | Serves as Study Coordinator for this project. Mr. Jones is responsible for coordinating the efforts of the co-investigators, acquiring all of the clinical data and making arrangements to obtain high-resolution CT (HRCT) scans, peripheral blood (DNA, RNA from PBMCs, and plasma), and pulmonary function tests (PFTs) on asymptomatic siblings of established IPF patients in Years 1-2 and the follow-up HRCT scans and PFTs in Years 3-4 on subjects with PrePF. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Rebecca Keith, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinate the accrual of patients and their families at the Denver VAMC |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Mark Steele, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 4 |
| **Contribution to project:** | Contribute to recruitment and accrual of patients and their families at University of Colorado and phenotype enrolled participants. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Daniel Kass, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinate the accrual of patients and their families at University of Pittsburgh. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Paul Wolters, MD** |

| | |
|---|---|
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinate the accrual of patients and their families at UCSF. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **James Loyd, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinate the accrual of patients and their families at Vanderbilt University. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Joa de Andrade, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinate the accrual of patients and their families at University of Alabama. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Ginger Reeves, MD** |
| **Project Role:** | Study Coordinator, UAB |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Serve as Study Coordinator at UAB to help recruit study subjects and perform the mucociliary clearance assays (technetium 99m sulfur colloid scans) in 45 study subjects. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

**Project 2**

| | |
|---|---|
| **Name:** | **Sean Colgan, PhD** |
| **Project Role:** | Co-Project Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Supervise overall research, design and conduct experiments, communicate results to scientific community |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Erica Alexeev, PhD** |

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|---|---|
| **Project Role:** | Postdoctoral Fellow |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Matthew Goldberg** |
| **Project Role:** | Professional Research Assistant |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 12 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Anthony Gerber, PhD, MD** |
| **Project Role:** | Co-Project Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Direct research team at National Jewish Health |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Sarah Sasse, PhD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Fabienne Gally, PhD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 5 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

**Project 3**

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|---|---|
| **Name:** | **Christopher Evans, PhD** |
| **Project Role:** | Project Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Design and execution of experiments using in vivo models on lung fibrosis and interpreting and presenting data. |

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| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Naoko Liu** |
| **Project Role:** | Professional Research Assistant |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 6 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Breanna Symmes, PhD** |
| **Project Role:** | Postdoctoral Fellow |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 12 |
| **Contribution to project:** | Design and conduct experiments |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

**Project 4**

| | |
|---|---|
| **Name:** | **Ivana Yang, PhD** |
| **Project Role:** | Project Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Design and execution of the study, the day-to-day functioning, trouble-shooting, integration, training, and long-term planning of the study |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Oliver Eickelberg, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Provide expertise in procedures and methods for isolation and study of specific cell populations from IPF lung explants and biopsies |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Melanie Königshoff, MD** |
| **Project Role:** | Co-Investigator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Provide expertise in Wnt signaling and lung regeneration following injury |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Eunjoo Kim, PhD** |
| **Project Role:** | Postdoctoral Fellow |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 12 |
| **Contribution to project:** | Breeding of Ift88-Krt5 and Arl13b-Krt5 CKO animals; i.t bleomycin, and i.n. H1N1 treatments; immunofluorescence analysis of animal tissue; hydroxyproline assays |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Yang Hui, PhD** |
| **Project Role:** | Postdoctoral Fellow |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 6 |
| **Contribution to project:** | Isolation of distal airway stem cell populations from human and mouse tissue, Wnt signaling assays |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Elizabeth Davidson** |
| **Project Role:** | Professional Research Assistant |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 3 |
| **Contribution to project:** | NHBE cell culture; immunofluorescence analysis on human and animal tissue |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

**Biostatistics Core**

| | |
|---|---|
| **Name:** | **Tasha Fingerlin, PhD** |
| **Project Role:** | Biostatistics Core Director |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Coordinates all activities of the Core, including assisting with study design for all projects, creating/maintaining data storage and analysis pipelines, supervising data analyses and assisting in the interpretation and dissemination of those analyses. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

| | |
|---|---|
| **Name:** | **Kelsey Anderson** |
| **Project Role:** | Computer Programmer |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 3 |
| **Contribution to project:** | Responsible for designing and implementing database structures that allow the individual projects to efficiently deposit and retrieve study data, as well as coordinating |

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|---|---|
| | the integration of systems in such a way as to preserve individual study features while allowing efficient integration of data across projects. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Sean Jacobson** |
| **Project Role:** | Biostatistician |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Responsible for day-to-day analytic activities for all projects, with duties determined by Dr. Fingerlin in response to investigator needs and priorities. Works directly with Dr. Fingerlin to implement summary reporting, project analyses and data reports for Project Directors and works with the computer programmer to develop the data sets and implement data cleaning and reporting algorithms. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

**Administrative Core**

| | |
|---|---|
| **Name:** | **David Schwartz, MD** |
| **Project Role:** | Administrative Core Lead |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 1 |
| **Contribution to project:** | Responsible for the scientific coordination, direction of research emphasis, and administrative activities of the Program |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

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|---|---|
| **Name:** | **Sarah Handley** |
| **Project Role:** | Administrator |
| **Research Identifier (e.g. ORCID ID)** | |
| **Nearest person month worked:** | 2 |
| **Contribution to project:** | Manage the fiscal and administrative aspects of the Program and coordinate matters with participating departments, the University of Colorado, the subcontract sites, and the Department of Defense. |
| **Funding support:** | *Complete only if the funding support is provided from other than this award* |

## a. Has there been a change in the active other support of the Site PI or senior/key personnel since the last reporting period?

Dr. Schwartz's VA Merit award ended on 12/31/2016 and there is a pending new proposal that was submitted in September 2018.

Dr. Yang's MPI R01DK100340 ended on 6/30/2017 and R21HL121572 also ended on 6/30/2017. Her support on R21ES023384 (Evans) ended on 12/31/2016. Since the original proposal, Dr.

Yang has new support on the following grants. These projects do not interfere with her work on this project.

**UH3OD023248 (Dabelea)**
09/30/18 – 08/31/23
0.3 calendar

**R01HL140357 (Maier/Yang)**
07/01/18-06/30/22
1.8 calendar

**R01HL138181 (Julian)**
04/01/18 – 3/31/22
0.6 calendar

**R01AI132476 (Barnes)**
02/01/18 – 1/31/23
0.6 calendar

**R01HL104608 (Barnes/Mathias/Kenny)**
01/01/18 – 11/30/22
0.6 calendar

Dr. Colgan has reduced his effort from 4.2 calendar months to 2.4 calendar months on **R37DK050189 (Colgan)** and **R01DK095491 (Colgan)**.

Dr. Fingerlin was named Biostatistical Core Director on **P01HL132821** (Voelker). She is a Co-Investigator on **R01HL136681** (O'Connor, Hamzeh, Koth, & Leach) and **R01HL140357** (MPI Maier & Yang). In addition, Dr. Fingerlin is a Co-Investigator on **U01HL138626** (MPI Seibold & Burchard). These projects, and the work involved, do not interfere with her work on this project.

### b. What other organizations were involved as partners?

Nothing to report

# 8. Special Reporting Requirements

None

# 9. Appendices

None