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TITLE: Molecular Profiles of Smokers with Subclinical Interstitial Lung Disease

PRINCIPAL INVESTIGATOR: George Washko

CONTRACTING ORGANIZATION: Brigham and Women's Hospital, Boston, MA

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Dr. George Washko, MD, Jr.

E-Mail:GWashko@BWH.Harvard.edu

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Brigham and Women's Hospital
15 Francis Street
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14. ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease poor survival, limited treatment options and no known cure. Recent groundbreaking clinical trials have shown that antifibrotic therapies can attenuate the decline in lung function particularly in IPF patients with mild interstitial lung disease (ILD). These remarkable findings support the concept that early detection of ILD could improve clinical outcomes by increasing secondary prevention opportunities. Accordingly, a recent NHLBI sponsored panel on the "Primary Prevention of Lung Disease" highlighted the need to better understand the natural history of early stages of pulmonary fibrosis in order to identify modifiable risk factors and in turn develop timely interventions to prevent disease progression. To address this important unmet need, the panel concluded that initial research efforts should focus on better defining phenotypic and molecular traits associated with subclinical ILD in susceptible populations.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease with poor survival, limited treatment options and no known cure. Recent groundbreaking clinical trials have shown that antifibrotic therapies can attenuate the decline in lung function particularly in IPF patients with mild interstitial lung disease (ILD). These remarkable findings support the concept that early detection of ILD could improve clinical outcomes by increasing secondary prevention opportunities. To address this important unmet need, the panel concluded that initial research efforts should focus on better defining phenotypic and molecular traits associated with subclinical ILD in susceptible populations.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

CT: Computed tomography; ILD: Interstitial Lung Disease; IPF: Idiopathic pulmonary fibrosis

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The Hypotheses and Specific Aims of our proposal are the following:
Hypothesis: In this proposal we will prospectively study a large cohort of smokers enrolled in the Pittsburgh Lung Screening Study (PLuSS). ***We hypothesize that select clinical and molecular features that portend a poor prognosis in IPF patients can predict development and progression of subclinical ILD in smokers.*** To test this hypothesis, we will address the following specific aims:
AIM 1: Determine prevalence and long-term progression of subclinical ILD in smokers enrolled in PLuSS. We hypothesize that interstitial abnormalities in a fibrotic subpleural distribution will be progressive while those with a centrilobular non-fibrotic distribution will remain
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Hypothesis: In this proposal we will prospectively study a large cohort of smokers enrolled in the Pittsburgh Lung Screening Study (PLuSS). ***We hypothesize that select clinical and molecular features that portend a poor prognosis in IPF patients can predict development and progression of subclinical ILD in smokers.*** To test this hypothesis, we will address the following specific aims:
AIM 1: Determine prevalence and long-term progression of subclinical ILD in smokers enrolled in PLuSS. We hypothesize that interstitial abnormalities in a fibrotic subpleural distribution will be progressive while those with a centrilobular non-fibrotic distribution will remain
stable or improve. We will perform an objective CT examination to assess progression, stability or regression of these changes and examine their clinical correlates.
Specific Aim 2: **Define clinical and molecular determinants that predict progression of subclinical ILD in smokers**. *We hypothesize that clinical and molecular determinants that portend a poor prognosis in IPF patients can predict progression of subclinical ILD.* We will demonstrate that select protein blood biomarkers (MMP7, CCL18 and SPD) that are known to predict disease

progression in IPF patients enhance the predictive ability of clinical features (Derived in Specific Aim 1) to identify ILA.

Specific Aim 3: Identify clinical and molecular determinants that predict the development of subclinical ILD in smokers. *We hypothesize those clinical and molecular determinants that predict disease progression in IPF patients can also predict the development of subclinical ILD in smokers.* We will demonstrate that clinical, radiologic and molecular determinants *(proteins, genetic determinants and DNA telomere length)* derived in Specific Aims 1, 2 and 3 can predict the development of subclinical ILD in smokers.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities: This was a two PI award. My group focused on the clinical and radiologic characterization of people at risk for developing parenchymal lung disease. Dr. Rosas and his team focused on the biologic bases of our observations. Our major focus of this award was to develop and implement image analysis tools that could detect and quantify interstitial changes in the lung tissue evident on CT scan as well as monitor the change of those features on serial images. To enable this, we first developed a deployed a method to mitigate the influence of image noise on our measures. This tool has now become part of a standard approach to monitoring disease activity in CT scan. We then measured the interstitial features in the baseline and longitudinal images available in our study cohorts and demonstrated that a combination of imaging, clinical and proteomics data could predict who will have progressive disease. Finally, we used these techniques in cohorts outside of what was proposed in this award to explore the metabolomic bases of lung disease as well as the association of these features with differential patterns of bronchial epithelial gene expression.

Specific Objectives: Our study related objectives were to develop and deploy an image analytic approach that allows the objective interpretation of low radiation dose CT scans. We then proposed to explore the clinical associations of the data we extracted from those scans. Finally, we sought to use those data as well as additional clinical and biologic information to identify people at greatest risk for the development of advanced parenchymal lung disease.

Significant Results/Key Outcomes: We successfully developed and deployed a suite of image analytic tools that enable objective assessment of lung disease in low radiation dose CT scans. We described their clinical relevance in peer reviewed literature and have now pivoted to exploring the biologic data available to use in cohorts both within and outside of our initial proposal. The most recent update to this work is described in detail in the final progress report submitted with this document.

The following is a detailed description of the tasks outlined in the initial SOW and the timeline for their completion. Note that this application included tasks for both Dr. Ivan Rosas (Initiating PI) and Dr. George Washko (Partnering PI). Only those tasks related to Dr. Washko's activities are listed herein.

Major Task 1 (Aim 1): To determine the prevalence and long-term progression of subclinical ILD in PLuSS subjects. There were several Subtasks within Major Task 1. These are briefly mentioned below and then addressed in aggregate.

A) Subtask #1: Complete transfer of PLuSS CT scans to Brigham and Women's Hospital – Complete
B) Subtask #2: Derive CT based metrics of parenchymal disease in all the baseline PLuSS CT scans – Complete.
C) Subtask #3: Transfer of baseline parenchymal data to Dr. Rosas' lab for integration into the PLuSS dataset – Complete.
D) Subtask #4: Derive CT based metrics of parenchymal disease in all the follow-up PLuSS CT scans – Complete.
E) Subtask #5: Transfer of longitudinal parenchymal data to Dr. Rosas' lab for integration into the PLuSS dataset – Complete.

These subtasks were all completed on the proposed timeline (within 36 months of the start of the award). The CT scans were transferred to Dr. Washko's lab for intake, quality control and organization. Given the high degree of noise in the computed tomographic images we retrained our image processing algorithm to generate a feature map of the lung tissue that may be free of some of the biases of low radiation dose CT scans. The complete reference for this work is provided below:

Vegas-Sanchez-Ferrero G, Ledesma-Carbayo MJ, Washko GR, San Jose Estepar R. Harmonization of Chest CT Scans for Different Doses and Reconstruction Methods. Med Phys. 2019; 46(7): 3117-3132.

An example of this noise mitigation technique applied to a CT scan in PLuSS is shown at the top of the next page.

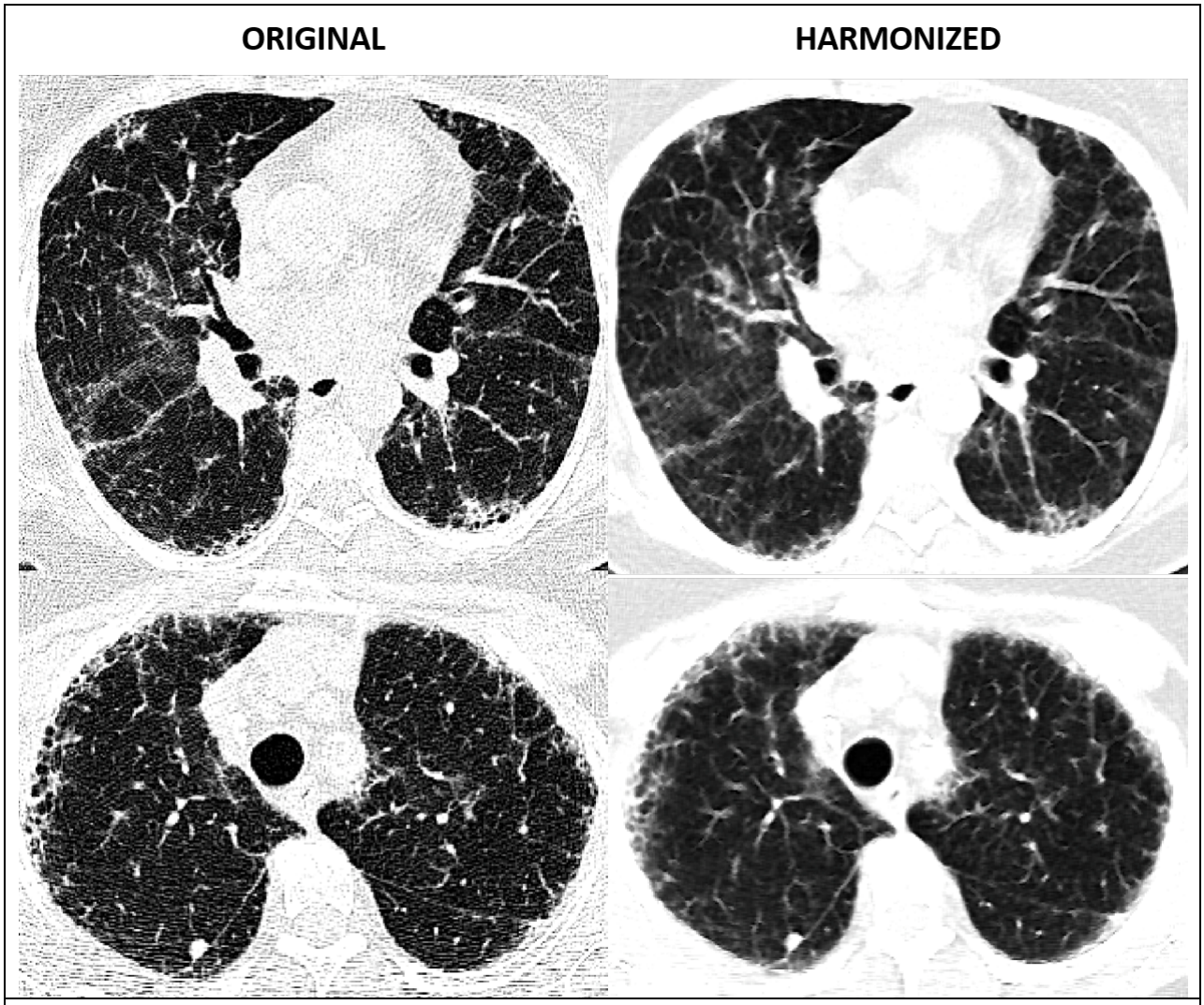

Figure 1: Side by side images before (left) and after (right) bias correction and noise reduction.

We also developed a comprehensive set of algorithms that label the features evident in lung tissue on CT scan. This deep learning-based approach to pattern recognition had the advantage that it was less sensitive to image noise and was more accurate for differentiating emphysema subtypes. A full reference for this work is provided below:

Bermejo-Pelaez D, Ash SY, Washko GR, San Jose Estepar R, Ledesma-Carbayo MJ. Classification of Interstitial Lung Abnormality Patterns with an Ensemble of Deep Convolutional Neural Networks. Scientific Reports. 2020;10(1):338.

An example of the application of this technique to a CT scan is provided in Figure 2 on the following page.

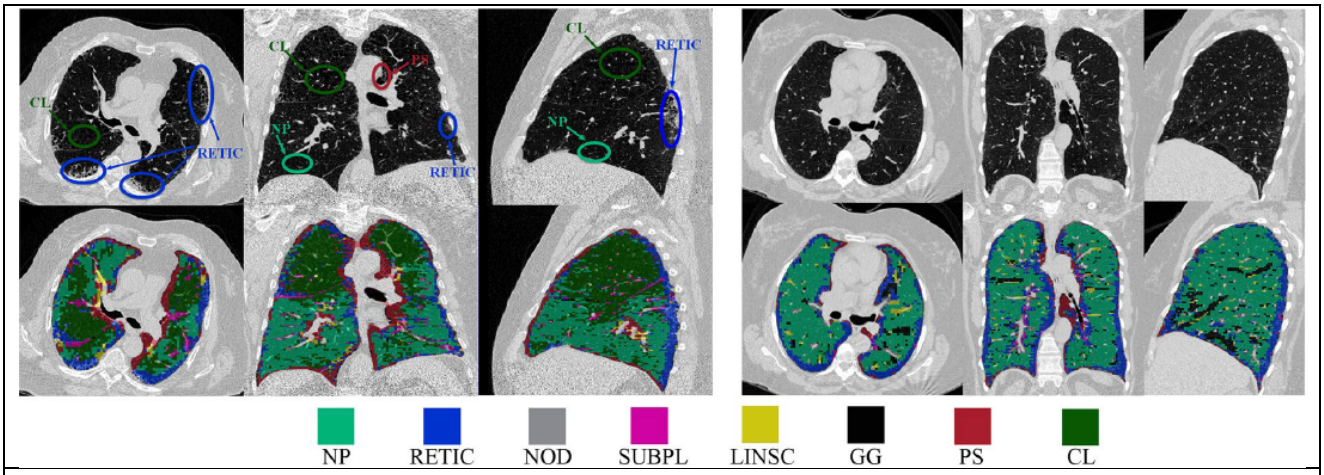

Figure 2: Automated full lung classification results for different patterns of interstitial change and disease severity (Figure 9 from Bermejo-Pelaez et al. Scientific Reports)

Following the development and deployment of robust methods to detect and quantify interstitial change on the CT scans of smokers, we sought to explore the clinical relationship of our radiologic findings. To do so we processed the longitudinal imaging data available in PluSS and COPDGene.

Clinical Associations:

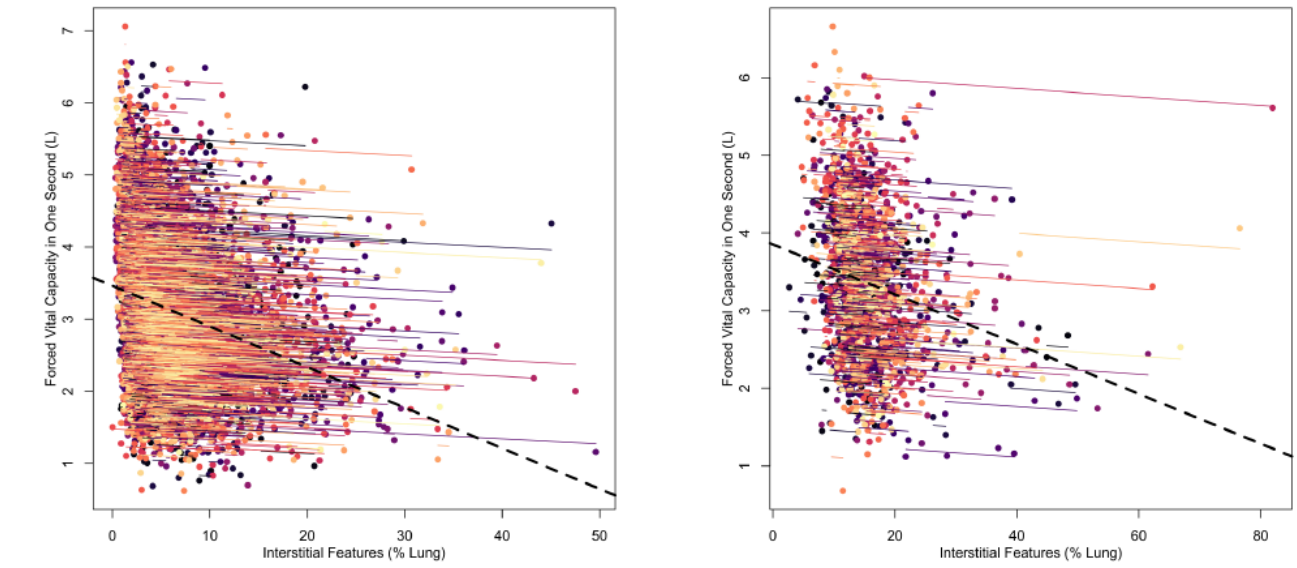

Figure 3: Plots showing the relationship between the change in interstitial features (X-axis) and change in Force Vital Capacity (FVC: Y-axis). Progression of parenchymal disease is associated with loss of lung volume and spirometric restriction in both COPDGene (left) and PLuSS (right).

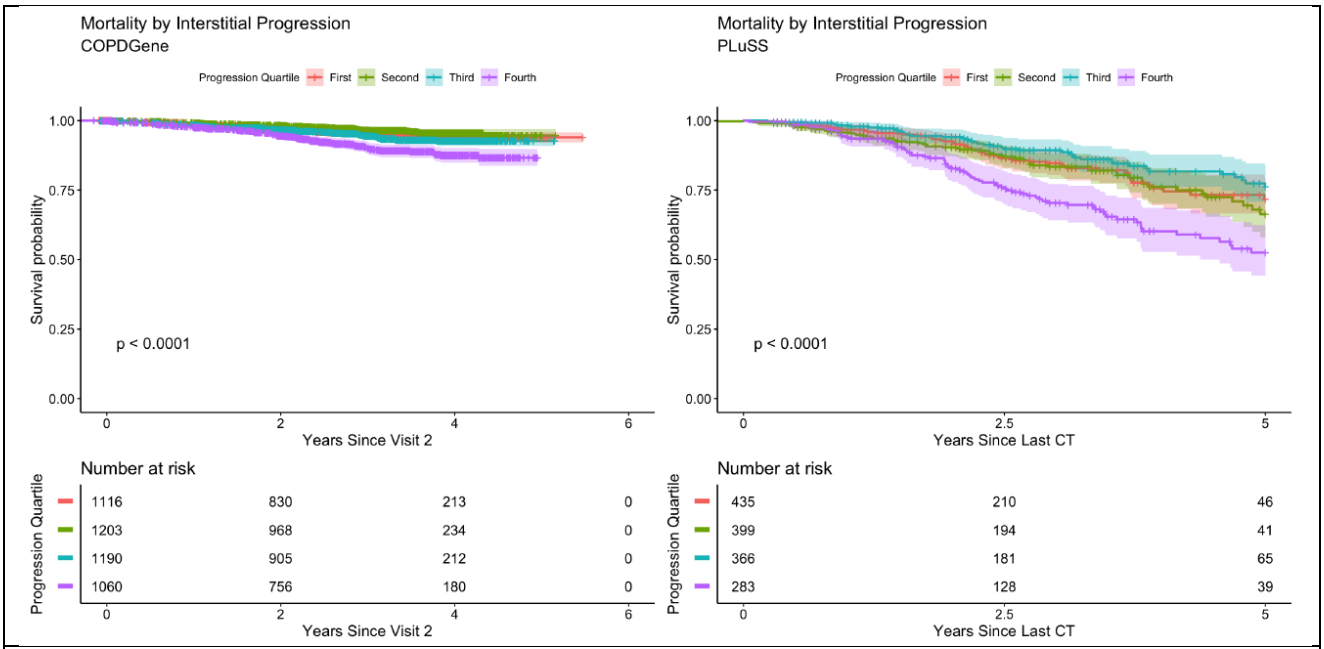

Figure 4: Relationship between progression of interstitial features and survival. Note that the fourth quartile represents those with the most progression. The figure to the left represents data from COPDGene and the figure to the right represents data from PLuSS.

These data suggest that we are detecting clinically relevant features in the CT scans of smokers and that progression (gain) of interstitial features are associated with poorer clinical outcomes (loss of lung function and increased risk of death). These data were published in Chest:

Choi B and colleagues. Quantitative Interstitial Abnormality Progression and Outcomes in the Genetic Epidemiology of COPD and Pittsburgh Lung Screening Study Cohorts. Chest. 2023 163(1):164-175.

Finally, our analyses demonstrate that progression of interstitial remodeling but not emphysema is associated with acute respiratory events. While this is a well-recognized complication in those with advanced interstitial lung disease/IPF, our analyses suggest that this condition may have episodes of accelerated progression even in its earliest stages. These data are provided in Table 1 on the following page. These data have been submitted for peer reviewed publication to the journal Radiology. We are now responding to a request for major revisions at that journal. A copy of this manuscript is provided in the Appendix to this final report (pages 100-148).

		Confidence Interval		
Phase	**Incident Rate Ratio**	**Lower**	**Upper**	**p**
Exacerbations between Phase 1 and Phase 2				
Change in Interstitial Features	1.09	1.02	1.18	0.018
Change in Emphysema	1.00	0.95	1.05	0.925
Exacerbations after Phase 2				
Change in Interstitial Features	1.07	1.00	1.15	0.036
Change in Emphysema	1.00	0.95	1.05	0.933

Table 1: Effect expressed as an increase in rate per 1 standard deviation increase in either interstitial features or emphysema based on multivariable zero-inflated negative binomial regression.

Finally, as also noted in previous annual progress reports, the excess noise of the PLuSS CT scans necessitated the development and deployment of an image analysis strategy that reduced artifact without affecting resolution. Our previous work described above focused on adjustment for noise and bias and we recently expanded this to include an adjustment for longitudinal changes in lung volume and its effect on lung features. This is detailed in the attached document (Standard Form 298) and is referred to as the Volume, Noise, and Bias (VNB) correction. This manuscript was just accepted for publication in the journal Radiology. It is titled "Quantification of Emphysema Progression at CT Using Simultaneous Volume, Noise and Bias Lung Density Correction" and is submitted with this final report (Appendix pages 1-28).

The aggregate of these published and unpublished data detail the completion of Subtasks #1-#4 of this Major Task. These published data also complete the two milestones of Major Task #1: (publication of the baseline (Milestone #1) and longitudinal (Milestone #2) PLuSS CT data). Finally, Dr. Rosas is a co-author and co-investigator of all of these studies and has received a copy of the CT scan data extracted from all of the PLuSS scans (Subtask #5).

Major Task #2 (Aim 2): To define clinical and molecular determinants that predict progression of subclinical ILD in smokers.

A) Subtask #1: Identify 300 ILA subjects and 300 age and sex matched controls – Complete
B) Subtask #2: Targeted biomarker analysis – Complete
C) Subtask #3: Discovery biomarker analysis using Somascan - Complete

We began this work by performing preliminary studies using the SomaScan assay for multiplexed blood-based proteomics. Our first step was to test this assay in a cohort of patients with IPF and healthy controls at Brigham and Women's Hospital.

Participants: IPF cases were reviewed and confirmed by thoracic radiologists at BWH. Biological samples and clinical information were obtained after informed consent and approved for use in this study by the institutional review boards at BWH (IRB Protocol 2001P000338). Peripheral blood samples were collected from subjects with IPF and Control and serum was stored in small aliquots at -80ºC. Clinical data and HRCT images closest to the date of blood sampling were extracted from the medical record.

Proteomics: The SOMAscan assay is a large-scale proteomics platform used for protein biomarker discovery and diagnostic and therapeutic development extensively described in prior publications. It utilizes single stranded oligonucleotide aptamers that bind protein with high affinity and specificity much like antibodies. The assay converts relative protein concentration into nucleic acid signals which are captured on streptavidin beads then quantified using DNA microarrays. The result is the relative abundance of the aptamer which is proportional to the amount of target protein in the sample expressed in relative fluorescence units (RFUs). The protein analytes include cytokines, chemokines, growth factors, and kinases and span a diverse range of secreted, intracellular and extracellular proteins. Quantification of 1321 proteins was performed using 150µl of serum from each of 75 subjects run simultaneously to avoid introducing non-biological variability from batch effects. Log base 2 transformation for normalization and inter-run calibration were performed according to standard quality control procedures. Raw

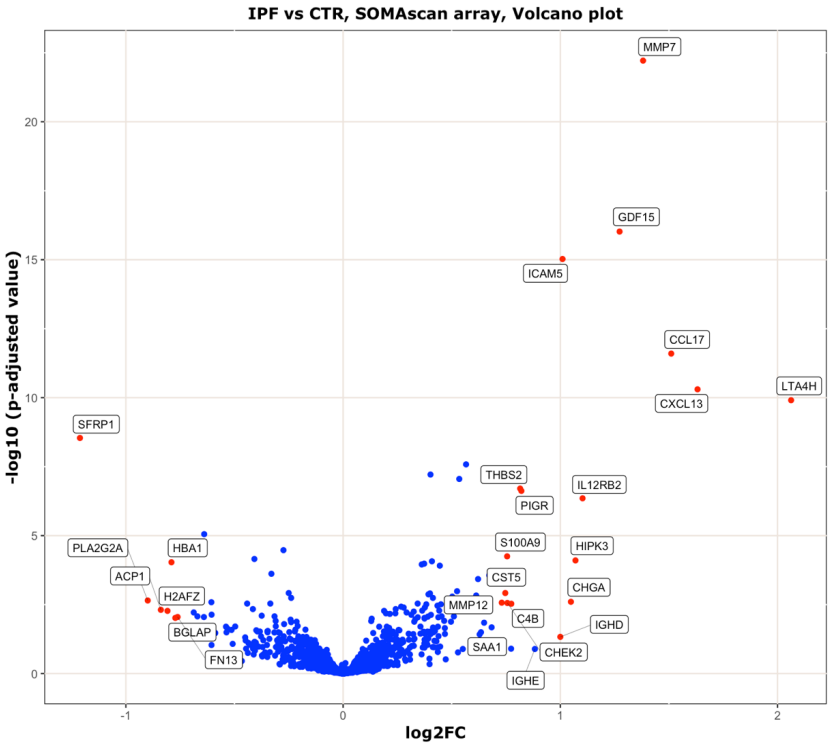

Figure 3: Statistically significant and biologically relevant proteins are shown in a volcano plot. The top 5 significant proteins that were over- in IPF with a log2FC >|1| included MMP7; 1.33 FC, LTA4H; 2.1 FC, GDF15; 2.90 FC, C-C motif chemokine ligand (CCL17; 1.69 FC), ICAM5; 1.06 FC.

SOMAscan data handling for further statistical analysis was done using readat package in R version 3.5.2. Protein intensities were fitted in a linear regression model adjusting for age and gender. Comparisons were performed with moderated t-test implemented by the limma Bioconductor package in the R environment to obtain log2 fold change and p values for each protein. P values were corrected for multiple comparisons using the Benjamini-Hochberg procedure with false discovery rate of 5%.

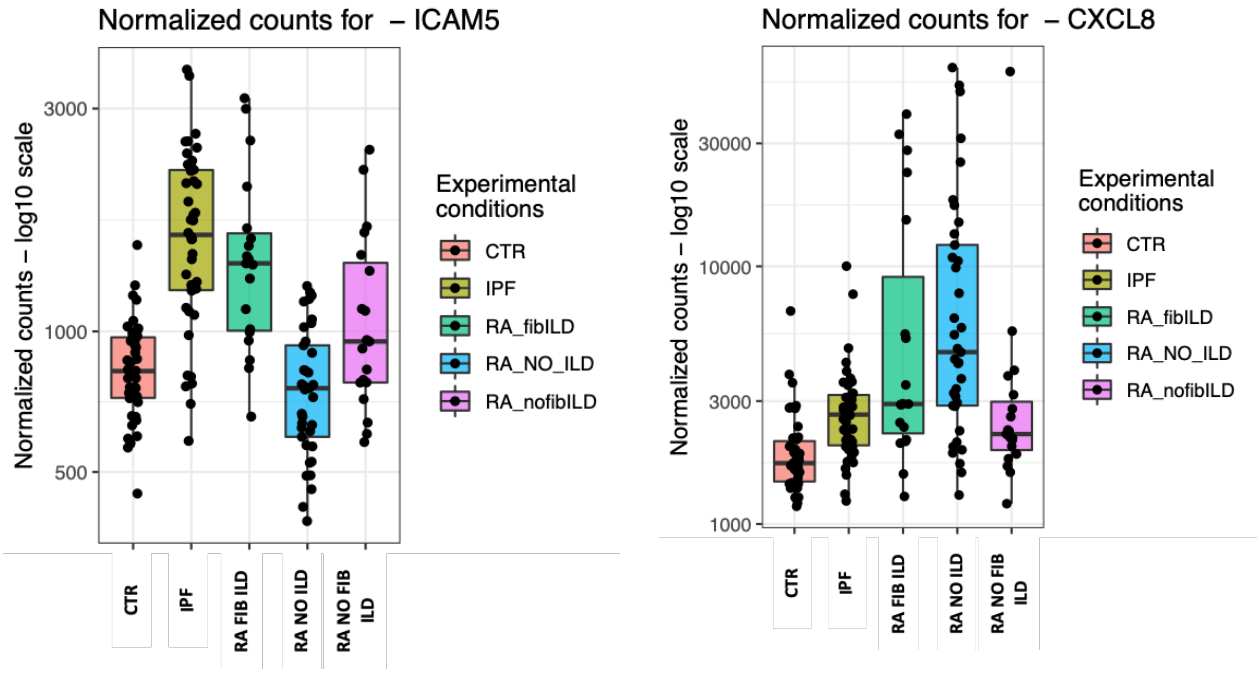

Figure 4: In a subsequent experiment, we compared a levels of select proteins in patients with IPF and patients affected with an inflammatory form of lung fibrosis found in patients with Rheumatoid Arthritis. We found that certain proteins are increased in IPF (i.e. ICAM5) whereas others biomarkers (i.e. CXCL8) are increased in an inflammatory form of fibrosis observed in patients with Rheumatoid Arthritis (RA ILD)

Taken together the preliminary findings of our proteomic studies using the SOMAscan demonstrate that select biomarkers are increased in IPF patients, it is reassuring to observe that the most upregulated biomarker is MMP7, which has been previously reported by our group in patients with IPF and families affected with subclinical ILD. In addition to known biomarkers, we also identified novel biomarkers increased in the peripheral blood of IPF patients. Additionally, we demonstrate that select biomarkers are increased in primary (IPF) and secondary (RA ILD) forms of lung fibrosis.

We then focused on the proteomic correlates of the lung features in two cohorts, COPDGene and PLuSS.

There are two manuscripts detailing the proteomics work in Major Task #2. The first is the attached manuscript: "Relationship Between Protein Biomarkers and Incident Interstitial Features in Smokers". This manuscript covers the proteomic discovery in COPDGene with replication and modeling in PLuSS and is attached to this final report – Appendix, pages 29-54. The second manuscript attached to this report titled "Proteomic Biomarkers of Quantitative Interstitial Abnormalities in COPDGene and CARDIA Lung Study" (Appendix, pages 55-82). This work focuses on proteomic discovery in COPDGene and the 5K Somascan platform followed by replication and pathways analyses in CARDIA. CARDIA is an NHLBI funded investigation to identify risk factors for cardiovascular disease and more recently risk factors for lung disease. It is a community-based population study and people have undergone serial examinations. Using the CT and proteomics data we demonstrated that the proteomic associations discovered in COPDGene largely replicate in a younger and much less smoke exposed cohort. This manuscript was submitted to the American Journal of Respiratory and Critical Care Medicine. It was returned for a request for major revision which we are currently completing. Figure 1 in the manuscript in the attached Appendix shows an example of the label map method for disease detection. Figures 2 and 3 of that manuscript in the Appendix illustrate the proteomics associations shared between COPDGene and CARDIA (2) and the correlations of the beta coefficients of the proteins between the two cohorts (3). Figures 4 and 5 depict the pathways analyses and deconvolution of the proteomic signatures to a range of organs in the body. Note that the lung was the highest score for both cohorts (Figure 5).

Metabolomics: We also explored the metabolomic correlates of our quantitative interstitial features in the COPDGene Study. Our work titled "Plasma metabolomics and quantitative interstitial abnormalities in ever-smokers" was just accepted to Respiratory Research. It is included in the appendix of this final report (pages 83-99). Please refer to Figure 3 for the metabolomic correlates of our quantitative features identified on CT scan. These data including the pathways enrichment are presented in tabular format (Tables 2, 3, and 4). **Major Task 3 (Aim 3)** is focused on the work that Dr. Rosas is completing in his laboratory.

Gene Expression Profiling: Finally, we examined the associations between QIA on CT scan and bronchial epithelial gene expression. To do this we leveraged data from the COPDGene Study and DECAMP. DECAMP (Detection of Early Lung Cancer in Active Military Personnel) was a Department of Defense (W81XWH-11-2-0161) investigation focused on the identification of biomarkers that could distinguish benign from malignant solitary pulmonary nodules in the lung as well as identify people at greatest risk for the development of future lung cancer. As part of that investigation, study participants underwent CT scanning of the chest and fiberoptic bronchoscopy and bronchial brushings. From these samples, bronchial epithelial gene expression data was generated for each person. The full reference for this publication is:

Billatos et al. Distinguishing Smoking-Related Lung Disease Phenotypes Via Imaging and Molecular Features. Chest. 2021; 159(2):549-563.

We began by creating three clusters in COPDGene based on CT features (preserved parenchyma, interstitial predominant, emphysema predominant) and exploring the clinical and epidemiologic characteristics of each group. An example of the CT images for each cluster are provided below in Figure 5.

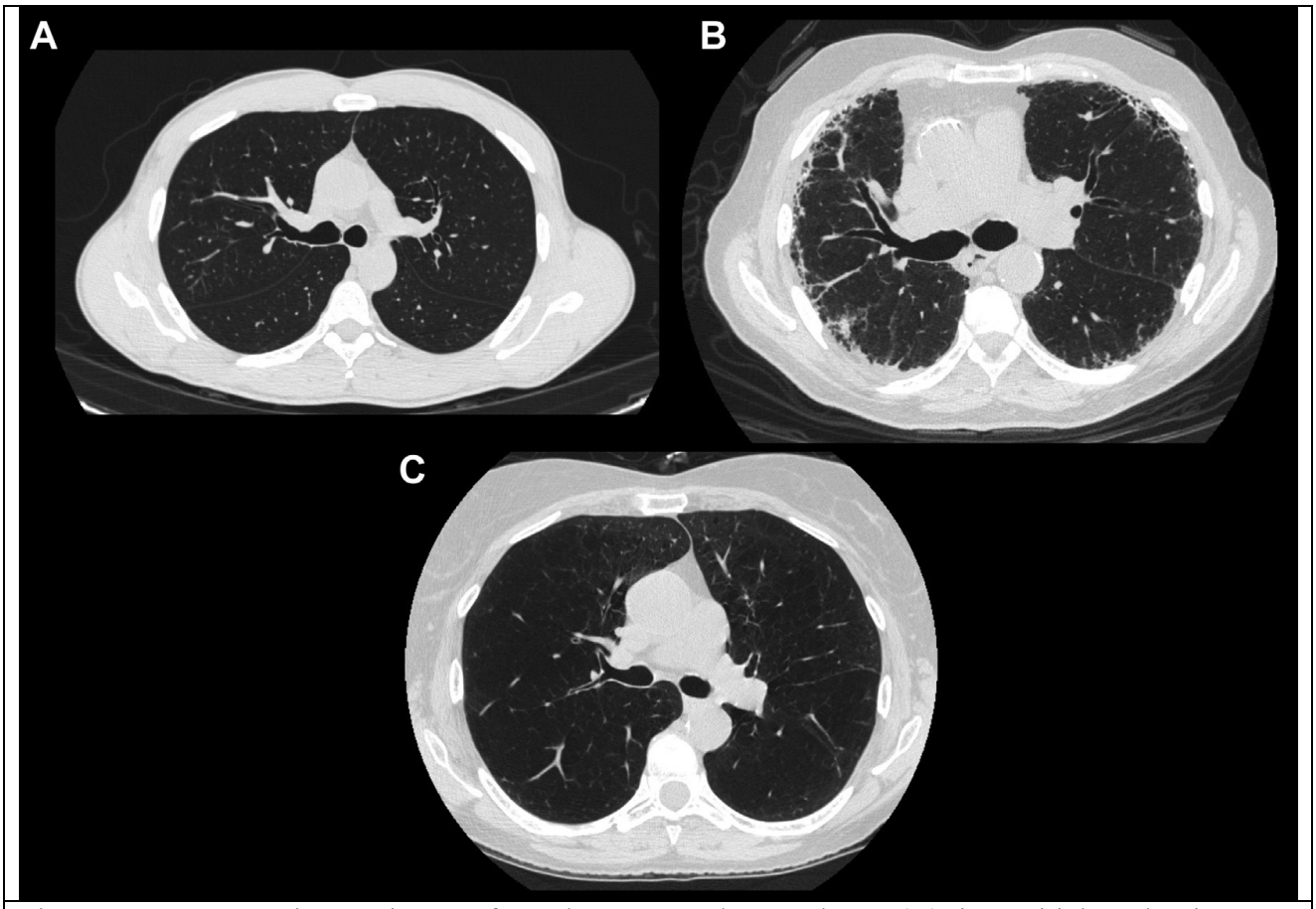

Figure 5: Representative CT images from the preserved parenchyma (A), interstitial predominant (B), and emphysema predominant (C). Taken from Billatos et al Chest 2021.

Finally, we focused on the differential bronchial epithelial gene expression by cluster. These data are provided on the top of the next page in Figure 6. Of note, samples from individuals in the interstitial cluster generally had intermediate levels of expression of these genes suggesting that at least some people may be in transition to a more emphysematous phenotype or that there is a shared mechanism of injury between the development of emphysema and interstitial remodeling of the parenchyma.

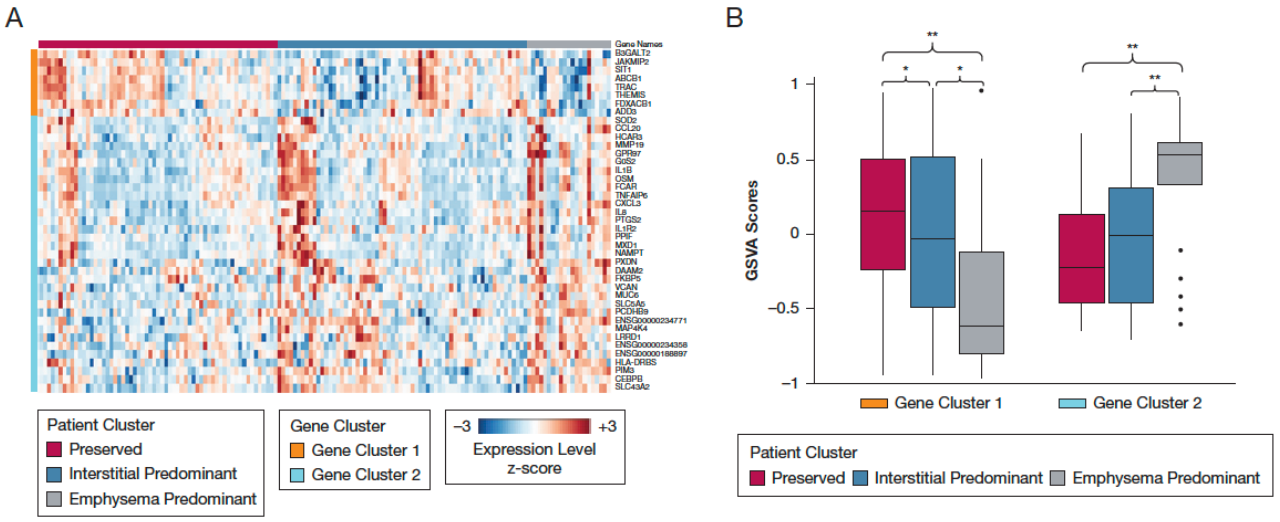

Figure 6: Emphysema cluster related gene expression (A). 41 genes were identified to be differentially expressed between the preserved and emphysema cluster. Gene set variation analysis was then used to summarize the expression of each gene cluster in each sample. Figure taken from Billatos et al. Chest 2021.

Summary: These subtasks were all completed within the timeline proposed in the initial SOW. There were 4 Milestones within Major Task #2 focused on the clinical utility of candidate biomarkers, the identification of novel biomarkers, the prognostic value of predictive biomarkers and manuscript preparation. Each of these four milestones within Major Task #2 have been completed as described above. Five manuscripts encompass this work. The first manuscript includes the data from Major Task #1 (prevalence, distribution and progression of interstitial features in PluSS – Choi et al. Chest 2023). The second manuscript is focused on the proteomic prediction of incident and progressive interstitial features (Appendix 29-54). The third manuscript describes discovery of new proteomic correlates of interstitial features and their biologic pathways. This work leveraged the SomaScan platform and consisted of discovery in COPDGene (n= 6,000) and replication in CARDIA (n=2,500). We were able to replicate our proteomics associations found in COPDGene even in the never-smoking subset of CARDIA (Appendix pages 55-82).The fourth manuscript explores the metabolomic associations of our quantitatively detected interstitial features evident on CT scan (Appendix pages 83-99). This work was external to PLuSS because of data availability and focused on the COPDGene Study. As described in the attached report, we found several novel metabolomics associations (both positive and negative) with CT data. The fifth and final manuscript mentioned above focused on the differential patterns of bronchial epithelial gene expression in controls, people with emphysema and people with interstitial changes on CT scan (Appendix pages 149-163).

The aggregate of these data strongly suggests that subtle interstitial features in the lung tissue on CT scan represent an early form of advanced lung disease. It is clear though that not all people with these QIA progress to fulminant respiratory disease. One remaining question to be answered relates to resilience. What are the biologic mechanisms that protect some people from this progression?

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project was not explicitly designed to support training and the professional development of junior faculty however several people in my group benefited from this ongoing work. This included Dr. Stefanie Mason, Dr. Samuel Ash, and Dr Bina Choi. Each person worked with me and our team of computer scientists on data interpretation and publication. The support of this award was instrumental in each person's career development.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The results were disseminated through peer reviewed publication as well as presentation in both national and international conferences. Finally, my team is dedicated to open-source science. My lab co-director, Dr. Raul San Jose Estepar developed and supports open-source software called the Chest Imaging Platform (CIP). This system has been downloaded by investigators around the world and was used and referenced in over 300 peer reviewed publications. We continue to transfer capabilities developed in this and our other Federal awards into the CIP for distribution.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report. This is the final report for this project.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This project provided the support to explore the use of image analysis software to detect people at greatest risk for future interstitial lung disease. To complete our proposed work, we leveraged our image analytic tools that can adjust for noise and detect underlying injury to the lung tissue. Using these data, we developed models using imaging, clinical and biomarkers data to identify smokers at greatest risk for having progressive interstitial lung disease.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

<u>Manuscripts accepted for publication during the award period:</u>

Vegas-Sanchez-Ferrero G, Ledesma-Carbayo MJ, Washko GR, San Jose Estepar R. Harmonization of Chest CT Scans for Different Doses and Reconstruction Methods. Med Phys. 2019; 46(7): 3117-3132.

Bermejo-Pelaez D, Ash SY, Washko GR, San Jose Estepar R, Ledesma-Carbayo MJ. Classification of Interstitial Lung Abnormality Patterns with an Ensemble of Deep Convolutional Neural Networks. Scientific Reports. 2020;10(1):338.

Billatos et al. Distinguishing Smoking-Related Lung Disease Phenotypes Via Imaging and Molecular Features. Chest. 2021; 159(2):549-563.

Choi B and colleagues. Quantitative Interstitial Abnormality Progression and Outcomes in the Genetic Epidemiology of COPD and Pittsburgh Lung Screening Study Cohorts. Chest. 2023 163(1):164-175.

Choi B, et al. Plasma Metabolites and Quantitative Interstitial Abnormalities in Ever-Smokers. 2023. Accepted for Publication.

Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, Chu SG, Raby BA, DeIuliis G, Januszyk M, Duan Q, Arnett HA, Siddiqui A, Washko GR, Homer R, Yan X, Rosas IO, Kaminski N. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. Sci Adv. 2020 Jul 8;6(28):eaba1983. doi: 10.1126/sciadv.aba1983. PMID: 32832599; PMCID: PMC7439502.

Maor Sauler, John E McDonough, Taylor S Adams, Neeha Kothapalli, Jonas S Schupp, Jessica Nouws, Maurizzio Chioccioli, Norihito Omote, Carlos Cosme Jr., Sergio Poli, Ehab A Ayaub, Sarah G Chu, Klaus H Jensen, Jose Gomez-Villalobos, Clemente J Britto, Micha SB Raredon, Pascal N Timshel, Naftali Kaminski, Ivan O Rosas. medRxiv 2020.09.13.20193417; doi: https://doi.org/10.1101/2020.09.13.20193417

<u>Imaging manuscripts submitted for publication during the award period:</u>

Choi B, Diaz AA, San Jose Estepar R, Enzer N, Castro V, Han MK, Washko GR, San Jose Estepar R, Ash SY for the COPDGene Study. Acute respiratory disease events associated with quantitative interstitial abnormality progression. Submitted for publication.

Ash SY, Doyle TJ, Choi B, Harmouche R, San Jose Estepar R, Castro V, Enzer N, Kalhan R, Reyfman PA, Liu GY, Bowler RP, Wilson DO, San Jose Estepar R, Rosas IO, Washko GR for the Pittsburgh Lung Screening Study and COPDGene Study Investigators. Relationship between Protein Biomarkers and Interstitial Feature Progression in Smokers. Submitted for publication.

Bina Choi, Gabrielle Y. Liu, Xiaoning Huang, Andrew Perry, Ruben San José Estépar, Samuel Y. Ash, Weihua Guan, David Jacobs, Fernando J. Martinez, Russell P. Bowler, Sadiya S. Khan, Raúl San José Estépar, Ravi Shah, Bharat Thyagarajan, Ravi Kalhan, George R. Washko. Proteomic biomarkers of quantitative interstitial abnormalities in COPDGene and CARDIA Lung Study

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

N/A

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

My group developed an image based technique to harmonize CT scan data across different levels of noise. We described this method in the progress reports. It has become a standard approach to perform longitudinal analysis in image based research of COPD progression.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

We are dedicated to making our work open-source. My team designed, built and supports the Chest Imaging Platform (CIP: www.chestimagingplatform.org)

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

George Washko M.D.

Project Role:	PI
Nearest person month worked:	3
Contribution to Project:	Dr. Washko was the PI of this investigation and was responsible for auditing data quality and exploring the clinical utility of the data generated.

Raul San Jose Estepar Ph.D.

Project Role:	Co-Investigator
Nearest person month worked:	2
Contribution to Project:	Dr. San Jose Estepar is the technical director of our lab and is responsible for building and maintaining the image analytic tools described in this and previous progress reports.

Stefanie Mason M.D.

Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to Project:	Dr. Mason was completing her fellowship training during the funding period of this award. She assisted in the collection, curation and analyses of the PLuSS data.

Samuel Ash M.D.

Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to Project:	Dr. Ash completed his fellowship training during the funding period of this award. He was primarily responsible for

	modeling the progression of the imaging data in PLuSS and discovering the proteomic predictors of that change.

Bina Choi M.D.

Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution to Project:	Dr. Choi is in her final year of fellowship training at Brigham and Women's' Hospital. She has led efforts at proteomic discovery of our quantitatively assessed interstitial features in the COPDGene and CARDIA cohorts.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

This is part of a partnering award with Dr. Ivan Rosas at Baylor College of Medicine. I have nothing further to report beyond that organization.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.