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14. ABSTRACT
Purpose: To test the hypothesis that the replacement of tissue-resident alveolar macrophages with monocyte-derived macrophages in response to repeated injury over the lifespan explains the delayed onset of lung fibrosis until the later decades of life. We will use our experimental findings in mice to guide an unbiased assessment of macrophage heterogeneity in normal human lungs and lungs from patients with pulmonary fibrosis. The overarching goal of these studies is to identify clinically applicable biomarkers that can guide therapy and factors released from tissue-resident macrophages or bone marrow-derived macrophages that prevent or promote fibrosis, respectively so they can be targeted for prevention or therapy.
Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice. Mice are currently being aged. The work is continuing according to SOW, no major findings to report. However, we have generated improved, more economical and robust mouse model to distinguish tissue-resident and monocyte-derived alveolar macrophages.
Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of accelerated pulmonary fibrosis. The work on this aim is in progress, in accordance with SOW, no major findings to report at the moment (expected in the year 2 of award).
Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with pulmonary fibrosis. We have recruited a planned number of patients into the study and performed transcriptional profiling by means of RNA-seq on single cell and bulk sorted populations or alveolar macrophages. Major findings: In agreement with our hypothesis, using single cell RNA-seq, we found substantial heterogeneity of alveolar macrophages in the healthy donor lung, we now sequencing and analyzing data from patients with lung fibrosis. In addition, we found that gene expression signature of profibrotic monocyte-derived alveolar macrophages generated in mouse models of lung fibrosis was present in the alveolar macrophages of patients with various forms of lung fibrosis.

15. SUBJECT TERMS
alveolar macrophages, pulmonary fibrosis

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Introduction

Purpose: To test the hypothesis that the replacement of tissue-resident alveolar macrophages with monocyte-derived macrophages in response to repeated injury over the lifespan explains the delayed onset of lung fibrosis until the later decades of life. We will use our experimental findings in mice to guide an unbiased assessment of macrophage heterogeneity in normal human lungs and lungs from patients with pulmonary fibrosis. The overarching goal of these studies is to identify clinically applicable biomarkers that can guide therapy and factors released from tissue-resident macrophages or bone marrow-derived macrophages that prevent or promote fibrosis, respectively so they can be targeted for prevention or therapy. This hypothesis will be tested in the following aims: Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice. Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of accelerated pulmonary fibrosis. Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with pulmonary fibrosis.

Keywords

Alveolar macrophages, pulmonary fibrosis, idiopathic pulmonary fibrosis

Accomplishments

Major Goal: Specific Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice.

Major Task 1: Are tissue-resident alveolar macrophages replaced by monocyte-derived alveolar macrophages during normal aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Aging of shielded bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Soberanes, Bartom and Chen).
Accomplishments: We have completed experiment related to this subtask. We have found that "healthy" aging, i.e. aging mice without lung injury, does not lead to the accelerated replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages. Moreover, using RNA-seq on FACSorted macrophages, we found a highly reproducible group of genes, differentially expressed between tissue-resident alveolar macrophages (originating from fetal monocytes) and monocyte-derived alveolar macrophages (originating from adult circulating monocytes) irrespective of age. This cluster of gene contained several transcription factors and pathogen recognition receptors, such as *Marco*. Both tissue-resident and monocyte-derived alveolar macrophages exhibited similar changes during the normal aging, characterized by the decreased expression of the cell cycle genes and increased expression of the components of immune-inflammasome. These findings informed our studies in Major Task 2. These findings were reported in our recent preprint: McQuattie et al., bioRxiv, 2019 (doi: 10.1101/717033).

Major Task 2: Do insults associated with normal aging accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Infect shielded chimeric mice with three strains of influenza A (A/WSN/33 and A/PR8/34 and A/Udorn/72) at 2, 4 and 6 months of age. Dr. Soberanes will perform the infections, Drs. Soberanes and Chen will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: As reported previously, generation of the shielded bone marrow chimeras is complete (100%). We have finished harvesting tissues from all experimental animals. Flow cytometric analysis demonstrates accelerated replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages. Moreover transcriptomic profiling of FACSorted tissue-resident and monocyte-derived alveolar macrophages via RNA-seq at multiple time points

demonstrated that lung injury can reprogram tissue-resident alveolar macrophages. These findings were reported in our recent preprint: McQuattie et al., bioRxiv, 2019 (doi: 10.1101/717033).

Major Task 3: Does replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages lead to exaggerated fibrosis in mice?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice on using CD11c-DTR mice as a host. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Initiate bleomycin-induced lung fibrosis in shielded chimeric mice and harvest macrophage populations. Dr. Soberanes will instill mice with bleomycin, Drs. Chen and Soberanes will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: As reported during the last year, during the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.
Using transcriptomic profiling of the FACSorted alveolar macrophages we have demonstrated that local environment (normal vs inflammatory milieu) during the niche repopulation plays a crucial role in programming monocyte-derived alveolar macrophages. While monocyte-derived alveolar macrophages recruited into non-inflammatory environment after sterile depletion with clodronate-loaded liposomes did not exhibited pro-fibrotic activity and behaved similarly to tissue-resident alveolar macrophages, monocyte-derived alveolar macrophages recruited during bleomycin-induced or influenza A-induced lung injury exhibited marked profibrotic activity, resulting in marked transcriptional changes (as determined by RNA-seq) as well as changes in lung function (as determined by direct measurement of lung mechanics with Flexivent). These findings were reported in our recent preprint: McQuattie et al., bioRxiv, 2019 (doi: 10.1101/717033).

Major Task 4: Can adoptive transfer of tissue-resident or monocyte-derived alveolar macrophages rescue or exacerbate, respectively, the severity of experimental lung fibrosis?
Subtask 1: Sort alveolar macrophages, generate bone marrow derived macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages and generate bone marrow derived macrophages in vitro. Dr. Soberanes will perform adoptive transfer of alveolar and bone marrow-derived macrophages and instill mice with bleomycin. Drs. Chen and Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for

RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes and Chen).
Accomplishments: We have established protocol for adoptive transfer of alveolar macrophages and performed initial experiments. Our data suggest that adoptive transfer of tissue-resident alveolar macrophages does not rescue experimental lung fibrosis. These findings were reported in our recent preprint: McQuattie et al., bioRxiv, 2019 (doi: 10.1101/717033).
We have recently **published** summary of our work for the Aim 1 of the current project in the Journal of Experimental Medicine (Misharin et al., JEM, 2017). We also presented our work at American Thoracic Society meeting in Washington, DC and 2017 MHSRS meeting in Florida. Importantly, our findings were independently confirmed by Dr. Janssen's group (McCubbrey et al., AJRCMB, 2017), which speaks of high reproducibility of our data. Another manuscript, summarizing the data on the role of local environment in programming tissue-resident and monocyte-derived alveolar macrophages in currently in preparation.

Specific Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of the accelerated pulmonary fibrosis.

Major Task 1: Do macrophages from young and aged mice differentially regulate chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice with tissue-resident or monocyte-derived alveolar macrophages using young and aged CD11c-DTR mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Sort tissue-resident alveolar macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages. Dr. Soberanes will perform adoptive transfer of alveolar and instill mice with bleomycin. Dr. Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Subtask 3: Bleomycin-induced lung fibrosis in bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Soberanes and Chen).
Accomplishments: See above, Aim 1, Major task 4. During the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce

repopulation with monocyte-derived alveolar macrophages. These findings were reported in our recent preprint: McQuattie et al., bioRxiv, 2019 (doi: 10.1101/717033).

Major Task 2: Does chronic stress in the epithelium accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages during aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages). Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific Cre^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep). These mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing these mice to Cre^{Sftpc} mice and initiated the proposed experiments. While this change of tools delayed the work on this subtask, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.
We have discussed our approach with Dr. Mary Armanios, one of the world's experts in telomerase deficiency-associated pulmonary fibrosis. This consultation has confirmed our current direction.
Experiments, conducted during the last quarter, demonstrated that young mice with deficiency of *Trf1* in alveolar epithelial cells exhibit enhanced response to profibrotic stimulus (bleomycin) and delayed recovery in response to influenza A infection. The manuscript describing the role of epithelial injury in macrophage programming is currently in preparation.
Subtask 2: Harvest and analyze the lungs. Harvest lungs from shielded chimeric at designated time points (6 weeks, 4, 12 and 18 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes).
Accomplishments: The work on this subtask was delayed because of the change in the mouse strain (see above). We have completed experiments using mice with *Trf1*-deficiency in alveolar type 2 cells. We also found that modulation of the epithelial stress using small molecule inhibitor of the integrated stress response ISRIB ameliorates recruitment of the profibrotic monocyte-derived alveolar macrophages. Some finding regarding the role of the local epithelial injury in the recruitment and maintenance of the profibrotic alveolar macrophages were reported in our recent publication (Joshi et al., Eur Resp Journal, 2019). The second manuscript describing the role of epithelial injury in macrophage recruitment and programming is currently in preparation.

Major Task 3: Can the adoptive transfer of tissue-resident alveolar macrophages improve chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. We found that adoptive transfer of tissue-resident alveolar macrophages does not improve pulmonary fibrosis. Instead, we found the way to modulate epithelial stress using small molecule inhibitor of the integrated stress response ISRIB. We found

that this intervention ameliorated recruitment of the monocyte-derived alveolar macrophages and decreased severity of pulmonary fibrosis.

Specific Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with IPF.
Major Task 1: Collect samples, perform RNA-seq on populations of lung macrophages, perform single cell RNA-seq and analyze the data.
Subtask 1: Submit documents for local IRB review. Organize workflow, establish standard operating procedures, meeting with all co-investigators.
Accomplishments: Our study protocol was approved by the local IRB on 2016/06/05. This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements. We received an initial note of approval on 2015/12/01 and HRPO Approval Memorandum on 2016/1/9, HRPO Log Number A-18899.b.
Subtask 2: Begin study. Recruit first patients to the study (Drs. Mutlu, Bhorade, Budinger). Perform pilot RNA-seq on populations of pulmonary macrophages, establish optimal workflow and data analysis pipelines (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).
Accomplishments: We have achieved a significant progress on this subtask. Since approval of the protocol by IRB and HRPO over 70 lung samples were processed, including donor lungs and lungs from patients with various forms of lung fibrosis (SSc-ILD, IPF, ILD, MCTD, hypersensitivity pneumonitis, pneumoconiosis, CPFE). As proposed, we have FACSorted alveolar macrophages and alveolar type 2 cells from these samples and extracted RNA for gene expression profiling using RNA-seq. In addition, to emphasize the advantages of the focusing on specific cellular populations we analyzed gene expression profiles from the whole lung tissue (traditional approach implemented by other groups). All samples have yielded high-quality (RIN over 7) RNA. Libraries for RNA-seq were prepared using a high-throughput automated robotic platform (Agilent Bravo) to minimize a batch effect, all libraries have passed the QC. Libraries from were sequenced on Illumina NextSeq 500 instrument. Data was processed using an established bioinformatic pipeline on high-performance computational cluster QUEST (Dr. Bartom). Integrative analysis of the whole lung tissue, alveolar macrophages and alveolar type 2 cells demonstrated that alveolar macrophages and alveolar type 2 cells carry transcriptional signature of pulmonary fibrosis and can be used for the diagnostics. These findings were reported in our manuscript: Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis, Am J Respir Crit Care Med. 2019;199(12):1517-36. doi: 10.1164/rccm.201712-2410OC.

Subtask 3: Single cell RNA-seq. Recruit patients to the study (Drs. Mutlu, Bhorade, Budinger). Process samples, generate single-cell RNA-seq libraries (Drs. Misharin, Chen, Soberanes). Perform RNA-seq, perform data analysis (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).
Accomplishments: We have recruited 17 subjects into the study: 9 subjects with various forms of pulmonary fibrosis, including 4 subjects with IPF, and 8 control subjects. Accordingly, we have performed single cell RNA-seq on these samples using 10x Genomics 3' V2 chemistry reagents. We have completed sequencing and analysis of all samples. We have identified several robust markers of profibrotic macrophages, including *SPP1*, *CHI3L1*, *MMP9* and others. We have validated these markers using fluorescent *in situ* RNA hybridization and immunohistochemistry.

These findings were reported in our manuscript: Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis, Am J Respir Crit Care Med. 2019;199(12):1517-36. doi: 10.1164/rccm.201712-2410OC. To provide easy access to the dataset for the larger scientific and public community we have developed an intuitive web-tool for the dataset exploration, which is available at https://www.nupulmonary.org/resources/.

Major Task 2: Design diagnostic flow cytometry panel.
Subtask 1: Select prospective markers based on RNA-seq data (Drs. Misharin, Budinger, Berdnikovs, Perlman). Screen antibodies for panel design (Dr. Misharin). Design and validate the panel (Drs. Misharin, Budinger).
Accomplishments: We have performed validation of the markers of profibrotic macrophages via fluorescent *in situ* RNA hybridization and immunohistochemistry and identified several candidate markers, including MEP1 and PLXND1. We are currently testing these reagents in the independent cohort.

Opportunities for training and professional development:

Impact on society beyond science and technology: We have provided an intuitive web-tool for the single-cell RNA-seq dataset exploration, which is available at https://www.nupulmonary.org/resources/. This tool facilitated dissemination of our fidnings beyond a specific scientific community and extended it to the public/customers.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report

Impact

Impact on the development of the principal discipline(s) of the project: Our findings regarding the existence of the profibrotic alveolar macrophages in patients with pulmonary fibrosis (Reyfman et al, AJRCCM, 2019), were independently confirmed by three other groups (Morse et al., ERJ, 2019; Habermann et al., bioRxiv, 2019; Adams et al., bioRxiv, 2019) demonstrating high rigor and reproducibility of our findings.

Impact on other disciplines: We have reported existence of the profibrotic alveolar macrophages in patients with pulmonary fibrosis (Reyfman et al, AJRCCM, 2019). Recently, similar population of fibrotic scar associated macrophages was reported in the patients with liver cirrhosis (Ramachandran et al., Science, 2019), suggesting that we have identified a conserved mechanism, that is relevant to multiple fibrotic diseases.

Impact on technology transfer: Nothing to report.

Impact on society beyond science and technology: Nothing to report.

Changes/Problems

Changes in approach:

No changes in approach since last progress report.

Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report.
Changes that had a significant impact on expenditures: Nothing to report.
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

Products

Journal publications:

1. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, Fernandez R, Akbarpour M, Chen CI, Ren Z, Verma R, Abdala-Valencia H, Nam K, Chi M, Han S, Gonzalez-Gonzalez FJ, Soberanes S, Watanabe S, Williams KJN, Flozak AS, Nicholson TT, Morgan VK, Winter DR, Hinchcliff M, Hrusch CL, Guzy RD, Bonham CA, Sperling AI, Bag R, Hamanaka RB, Mutlu GM, Yeldandi AV, Marshall SA, Shilatifard A, Amaral LAN, Perlman H, Sznajder JI, Argento AC, Gillespie CT, Dematte J, Jain M, Singer BD, Ridge KM, Lam AP, Bharat A, Bhorade SM, Gottardi CJ, Budinger GRS, Misharin AV. Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis. Am J Respir Crit Care Med. 2019;199(12):1517-36. Epub 2018/12/18. doi: 10.1164/rccm.201712-2410OC. PubMed PMID: 30554520; PMCID: PMC6580683

2. Joshi N, Watanabe S, Verma R, Jablonski RP, Chen CI, Cheresh P, Markov NS, Reyfman PA, McQuattie-Pimentel AC, Sichizya L, Lu Z, Piseaux R, Kirchenbuechler D, Flozak AS, Gottardi CJ, Cuda CM, Perlman H, Jain M, Kamp DW, Scott Budinger GR, Misharin AV. A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signaling in monocyte-derived alveolar macrophages. Eur Respir J. 2019. Epub 2019/10/12. doi: 10.1183/13993003.00646-2019. PubMed PMID: 31601718.

3. McQuattie-Pimentel AC, Ren Z, Joshi N, Watanabe S, Stoeger T, Chi M, Lu Z, Sichizya L, Piseaux R, Chen C-I, Soberanes S, Reyfman PA, Walter JM, Anekalla KR, Davis JM, Helmin KA, Runyan CE, Abdala-Valencia H, Nam K, Meliton AY, Winter DR, Morimoto RI, Mutlu GM, Bharat A, Perlman H, Gottardi CJ, Ridge KM, Chandel NS, Sznajder JI, Balch WE, Singer BD, Misharin AV, Budinger GS. The Aging Microenvironment Shapes Alveolar Macrophage Identity in Aging. bioRxiv. 2019:717033. doi: 10.1101/717033.

Presentations:

1. Alexander Misharin. Using the map: single-cell RNA-seq provides mechanistic insights into the role of macrophages in pulmonary fibrosis. Novartis Institute For BioMedical Research, Boston, MA, USA. June 10, 2019.
2. Alexander Misharin. The Human Lung Cell Atlas Seed Network. HuBMAP Common Coordinate Workshop. Harvard Medical School. Boston, MA, USA. June 27, 2019.
3. Alexander Misharin. Alveolar macrophages as orchestrate pathogenesis of pulmonary fibrosis. Genentech Immunology and Infectious Diseases seminar series. South San Francisco, CA, USA. October 28, 2019.

Websites: We have provided an intuitive web-tool for the single-cell RNA-seq dataset exploration, which is available at https://www.nupulmonary.org/resources/. This tool facilitated dissemination of our fidnings beyond a specific scientific community and extended it to the public/customers.

Participants and other collaborating organizations

During the last annual period:
Name: **GR Scott Budinger**
Project Role: Principal Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2 Calendar Month
Contribution to Project: Dr. Budinger has prepared IRB protocol and worked on its revisions. He supervised generation of the shielded bone marrow chimeras and development of the new fate-mapping mouse models. He performed acquisition of the human lung samples for FACSorting, interpretation of RNA-seq results and presented data at the conferences.

Name: **Raul Piseaux**
Project Role: Postdoctoral Researches
Researcher Identifier: NA
Nearest person month worked: 2.3 Calendar Month
Contribution to Project: Dr. Piseaux monitored shielded bone marrow chimeras, managed mouse colony and worked on generating new fate-mapping mouse models.

Name**: Nikita Joshi**
Project Role: Postdoctoral Researcher
Researcher Identifier: NA
Nearest person month worked: 0.8 Calendar Month
Contribution to Project: Dr. Joshi worked on optimizing cell RNA-seq using Drop-seq technique. She processed human lung samples, generated and sequenced single cell RNA-seq libraries, and performing analysis of the dataset. She has also contributed to generation and analysis of the RNA-seq data from "bulk"-sorted alveolar macrophages.

List of all participants for this project:
Bartom,Elizabeth Thomas
Berdnikovs,Sergejs
Bhorade,Sangeeta Maruti
Budinger,Gregory Robert Scott
Joshi,Nikita Ramesh
Matsuda,Hiroaki
Piseaux Aillon,Raul
Runyan,Constance E

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

Other organizations involved as partners:

Organization Name: University of Chicago, Section of Pulmonary and Critical Care Medicine, Dr. Gokhan Mutlu
Location of Organization: Chicago, IL
Partner's contribution to the project: Dr. Mutlu provided us with human lung specimens for to fulfill the research as described in Aim 3.

Special Reporting Requirements: Nothing to report.

Appendices
Nothing to report.