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TITLE: MIF-Based Therapies in Cigarette Smoke-Related COPD and Pneumonia

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14. ABSTRACT

Chronic obstructive pulmonary disease (COPD) is the 3rd leading cause of death worldwide and is especially common in military members and Veterans. A major cause of mortality in people with COPD is bacterial pneumonia caused by *Streptococcus pneumoniae* (*S. pneumoniae*). We identified an innate immune protein, Macrophage Migration Inhibitory Factor (MIF) and it receptor, CD74, as endogenous, protective molecules that determine susceptibility to COPD and immunity against *S. pneumoniae*. Augmenting MIF levels in susceptible individuals may be effective therapy against COPD as well as *S. pneumoniae* infection. We have already developed orally active MIF agonists (MIF20) with excellent safety and efficacy profiles that are ready to be tested in COPD and bacterial pneumonia models. **Our overall objective of this proposal is to test MIF augmentation against CSE-related COPD and its subsequent complication, *S. pneumoniae*.** We propose to complete the following two **Aims: 1)** Test the therapeutic efficacy of MIF augmentation in CSE-related COPD; **2)** Test the therapeutic efficacy of MIF augmentation in CSE-related *S. pneumoniae* infection. These studies will provide pre-clinical testing of MIF agonist-based therapy in COPD and bacterial pneumonia.

15. SUBJECT TERMS

Lungs, COPD, emphysema, cigarette smoke, pneumonia, macrophage inhibitory factor, inflammation, immunity, therapy

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

COPD is a progressive, destructive process of airflow obstruction, leading to respiratory failure. A major cause of mortality in people with COPD is bacterial pneumonia, such as those caused by *Streptococcus pneumoniae* (*S. pneumoniae*). The main purpose of this research project is to perform pre-clinical therapeutic testing of MIF augmentation strategies, leveraging our recently-developed small molecules, in a mouse model of human COPD and bacterial pneumonia.

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

Lungs, COPD, emphysema, cigarette smoke, pneumonia, macrophage inhibitory factor, inflammation, immunity, therapy

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.

Major Goals/Milestones:

1. HRPO & ACURO Approval: 100% completed
2. Yale IRB/IACUC Approval: 100% completed
3. Dose selection using 3 doses and 3 routes of delivery (oral, systemic and intra-tracheal) and check for overt toxicity after 1 month. 100% completed
4. 1 dose selected and no toxicity detected: 100% completed
5. Increased activation and MIF-related proteins after MIF20 administration: 100% completed
6. Decreased injury and inflammation after MIF20 administration: 80% completed
7. Therapeutic effect after short-term smoke exposure: 60% completed
8. Therapeutic effect after long-term smoke exposure: 20% completed
9. Therapeutic effects of concurrent and post long-term smoke exposures: 20% completed
10. Therapeutic effect after *S. pneumoniae* infection following smoke exposure: not started yet

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.

This reporting period allowed pre-clinical, *in vivo* testing of MIF20, a MIF agonist, on pulmonary function, inflammation, and cellular senescence in short- and, in part, long-term cigarette smoke (CS) exposed mice (experimental design is shown in Figure 1). Major activities included testing of the protective effects of MIF20 against CS-induced lung dysfunction in both short-term (3 months; mainly, inflammation and apoptosis) and pilot long-term (6 months). These results are shown in Figures 1 – 10. To increase the robustness of our MIF20 testing, we studied 50% male and 50% female mice for all experiments. We found that male mice were more susceptible to CS-induced emphysema, indicated by the upward-shift of PV loops. Yet, MIF20 attenuated CS-induced emphysema only in female mice, as shown in Figure 3. Based on the short-term CS experiments, we selected 100 mg/kg as the optimal dose for MIF20 treatment in long-term CS experiments. We are still in the process of completing the long-term CS exposures and depending on the results, especially if the gender effect persists, we may need to re-address the optimal dosing issue. We also plan to perform post-treatment testing of MIF20 after long-term CS. During the past reporting period, we also performed dose-selection and infection-time periods for the *S. pneumoniae* lung infection 2-hit model (*S. pneumoniae* infection following CS exposure). These data are shown in Figure 11.

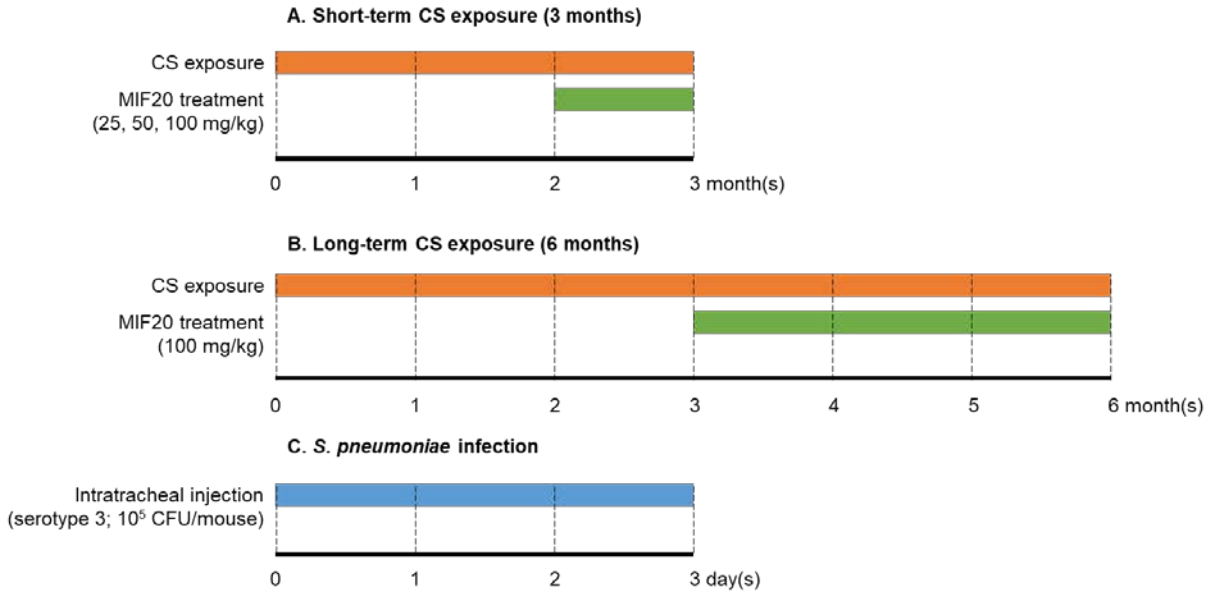

Figure 1. Experimental details. Animals: C57BL/6J mice (female and male, 6 weeks old) were supplied by Jackson Laboratory (Bar Harbor, ME, USA). The animals were housed in cages located in temperature- and humidity-controlled rooms with a 12 h light-dark cycle and received water and food *ad libitum*. **Cigarette smoke (CS) exposure**: The mice were exposed in a Teague machine to CS from scientific reference cigarettes (3R4F, University of Kentucky, Lexington, KY) at 140 mg/m^3 total suspended particulates for 3 or 6 months (6 h/day, 5 days/week). **MIF20 administration**: Mice were orally administered vehicle (1% carboxymethyl cellulose in water) or 25, 50, 100 mg/kg MIF20 once a day during the last 1 or 3 months of short- and long-term CS exposure, respectively. Body weights were recorded during MIF20 treatment to monitor any toxicity from long-term treatment of vehicle (1% carboxymethyl cellulose) and/or MIF20. ***S. pneumoniae* infection**: C57BL/6 mice were infected intratracheally with serotype 3 Sp (10^5 cfu per mouse) to establish a model of acute pneumonia. Infected mice were monitored for weight changes and euthanized at 3 days post-infection. Bronchoalveolar lavage (BAL) and type I cytokine induction were assessed. **Pulmonary function test (PFT)**: At the end CS exposure, mice were anesthetized by urethane and paralyzed with a pancuronium bromide (intraperitoneal injection). After confirming complete paralysis, the lungs were ventilated with a Constant Flow ventilator using the FlexiVent software to measure pressure-volume (PV) loop, lung compliance, resistance, elastance, and other pulmonary functional parameters. Bronchoalveolar fluid and lung tissues were collected, and then left lung was inflated with 1% agarose, fixed in 10% neutral formalin, and stained with H&E for further study. **Trichrome stain**: The lung tissues were collected and fixed in 10% normal formalin. Next day, tissues were transferred to 70% cold-EtOH, embedded in paraffin, sectioned, and stained with trichrome to detect collagen deposition. **Real-time RT-PCR**: Total RNA was extracted from lung tissues using TRIzol reagent according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). First-strand cDNA was synthesized using iScript (Bio-Rad, Hercules, CA). Real-time RT-PCR reactions were carried out with the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and an ABI Prism 7000 Sequence Detection System (Applied Biosystems). *gapdh* was amplified as a control. Real-time PCR conditions were 95˚C for 10 min and 40 cycles of 95˚C for 15 s, followed by 60˚C for 1 min. The relative quantification values for these gene expressions were calculated from the accurate threshold cycle (CT). Primers (5'→3') used for RT-PCR were follows. *p16* forward: CCCAACGCCCCGAACT, reverse: GCAGAAGAGCTGCTACGTGAA; *p19* forward: GGAGCTGGTGCATCCTGACGC, reverse: TGGCACCTTGCTTCAGGAGCTC; and *gapdh* forward: CATCACTGCCACCCAGAAGACTG, reverse: ATGCCAGTGAGCTTCCCGTTCAG. **Statistical analysis**: All data were analyzed by the student *t*-test or one-way ANOVA for post-hoc comparisons. Statistical differences between the groups were considered significant at $p<0.05$.

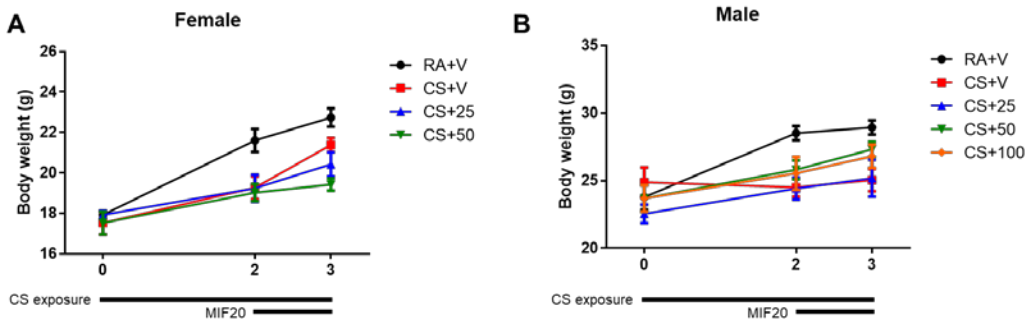

Figure 2. The effect of short-term cigarette smoke (CS) exposure and MIF20 treatment on body weight. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week), and were orally administered vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. The body weight of (A) female and (B) male mice were measured during vehicle or MIF20 treatment. After 3 months CS exposure, CS groups showed decreased body weight in both female and male mice. There was no significant differences between vehicle- and MIF20-treated group in CS-exposed mice. The data are shown as mean ± SEM (n=5 per group). RA=room-air.

Analysis/Interpretation: Given that loss of body weight is directly correlated to the LD_{50} (Toxicol Appl Pharmacol, 1968), body weight is considered as the gold standard in toxicology research as a sign of general toxicity. In the present study, we monitored near-moribund states, changes in body weight and gross observation for 28 days during MIF20 treatment. During the 3 months CS exposure, there was no mortality. The body weights detected in RA group mice corresponded well to the body weight ranges of age-matched normal mice (http://www.jax.org). CS exposure induced body weight loss in both female and male mice. MIF20 treatment did not affect the body weight throughout the groups.

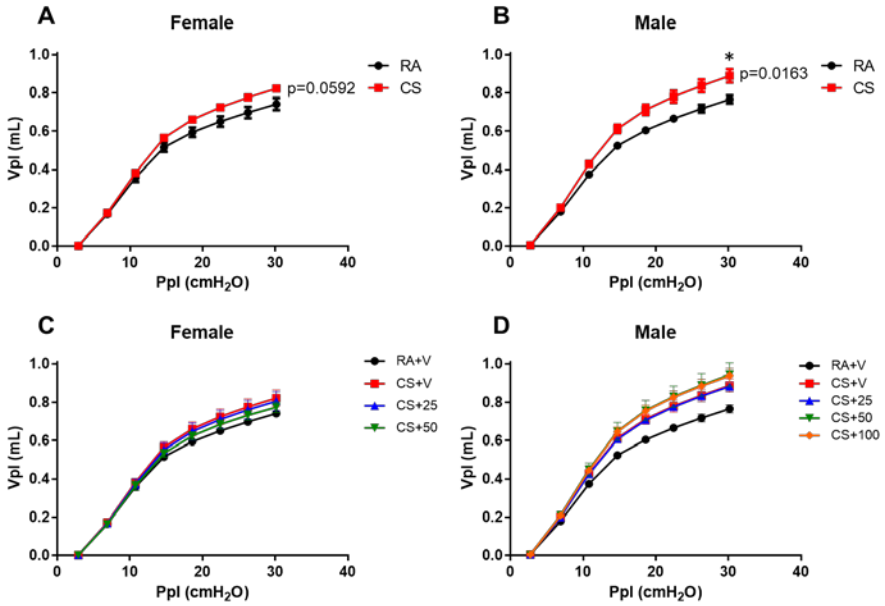

Figure 3. The effect of MIF20 on pressure-volume (PV) loops in short-term cigarette smoke (CS)-exposed mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). After anesthesia and paralysis the mice, PV loops were measured by FlexiVent (Scireq, Montreal, Canada). (A and B). CS exposure induced upward-shift of PV loops in both female and male mice compare to those of room-air (RA) group. (C) Female and (D) male mice were orally treated vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. The data are shown as mean ± SEM (n=5 per group). *p<0.05 vs. RA group.

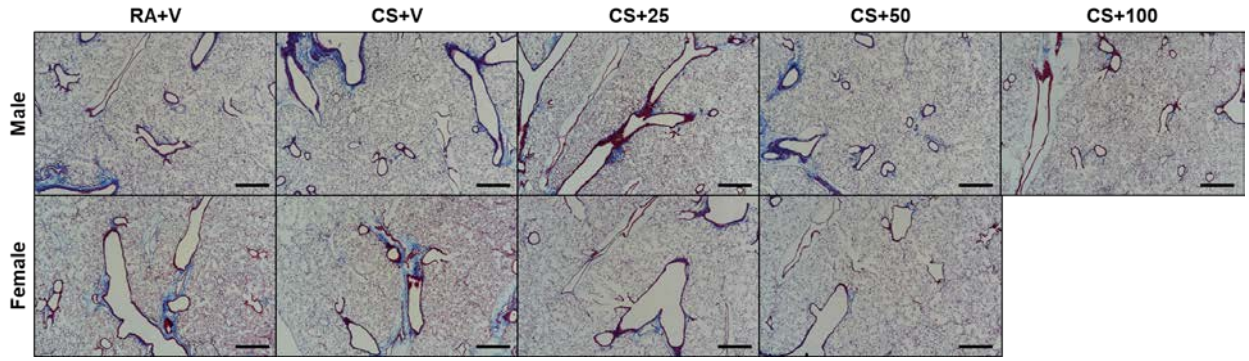

Figure 4. The effect of MIF20 on collagen deposition in short-term cigarette smoke (CS)-exposed mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). After anesthesia and paralysis, mouse lung tissues were collected and fixed in 10% formalin. The tissue sections were embedded in paraffin, sectioned, and stained with trichrome to detect collagen deposition. Scale bar = 100 μm.

Analysis/Interpretation: PV loops demonstrate how compliance changes, an indication of emphysema (or fibrosis), and has been regarded as the gold standard tool for assessment of the mechanical properties of the lungs after CS. Most of the early studies describing the relationship between PV loops and COPD were done to diagnose and establish the severity of emphysema, a major histopathologic and biomechanical feature of COPD. In the present study, 3 months CS exposure induced an upward-shift of PV loops in both female and male mice, which likely represents emphysematous lung. Male mice showed more distinct changes in PV loops than that the female mice. MIF20 treatment attenuated CS-induced upward-shift of PV loops in dose-dependent manner in female mice. Thus, we decided to select 100 mg/kg of MIF20 as the optimal dose for long-term CS exposure experiments. Of note, MIF20 treatment augmented CS-induced upward-shift of PV loops in dose-dependent manner in male mice. To determine whether there is fibrosis-like changes in the lungs of CS-exposed mice, we performed trichrome stain. As shown in Figure 4, there were no changes in collagen deposition in CS-exposed mice, so we were unable to establish overt fibrosis. However, more sensitive markers of lung fibrosis (hydroxyproline, e.g), will be necessary. Interestingly, MIF20 treated-group showed decreased trichrome-positive regions compared to RA+V and CS+V groups, which suggests a new potential role of MIF20 in lung fibrosis – which is beyond the scope of our currently funded studies but may launch new, future studies.

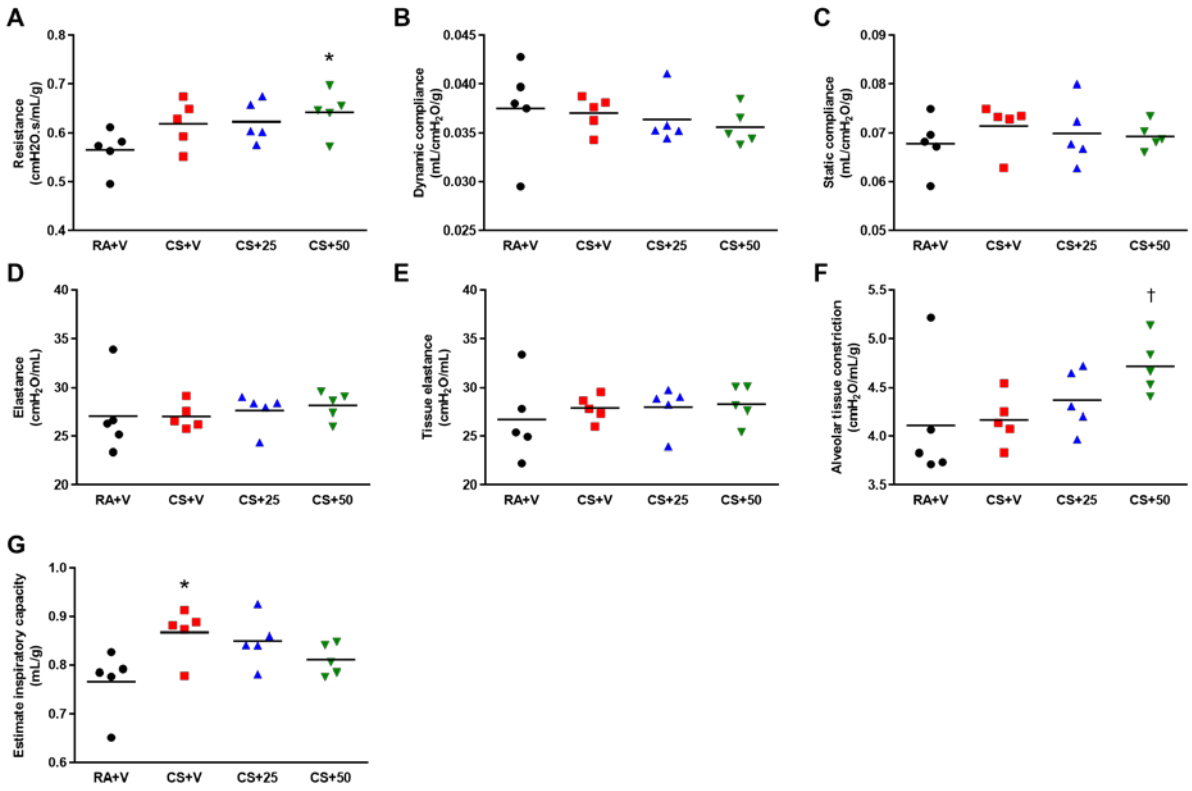

Figure 5. The effect of MIF20 on pulmonary function in short-term cigarette smoke (CS)-exposed female mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). Mice were orally treated vehicle (V), 25 or 50 mg/kg of MIF20 during the last 1 month. After anesthesia and paralysis, the mice were subjected to the following lung measurements, (A) resistance, (B) dynamic compliance, (C) static compliance, (D) elastance, (E) tissue elastance, (F) alveolar tissue constriction, and (G) estimate inspiratory capacity were measured by Flexivent (Scireq, Montreal, Canada). The data are shown as mean ± SEM (n=5 per group). *p<0.05 vs. RA+V group. †p<0.05 vs. CS+V group. RA=room-air.

Analysis/Interpretation: Lung compliance and estimate of inspiratory capacity are increased in emphysematous lung, while lung resistance, elastance and alveolar tissue construction are decreased in emphysema. Three months CS exposure increased estimate inspiratory capacity, suggesting increased early emphysema, and this increase was attenuated by MIF20 treatment in a dose-dependent manner. The lack of changes in resistance after MIF20 is reassuring, because this suggests that airway obstruction is not occurring. However, the other measures of emphysema were unchanged – likely due to the short CS time period.

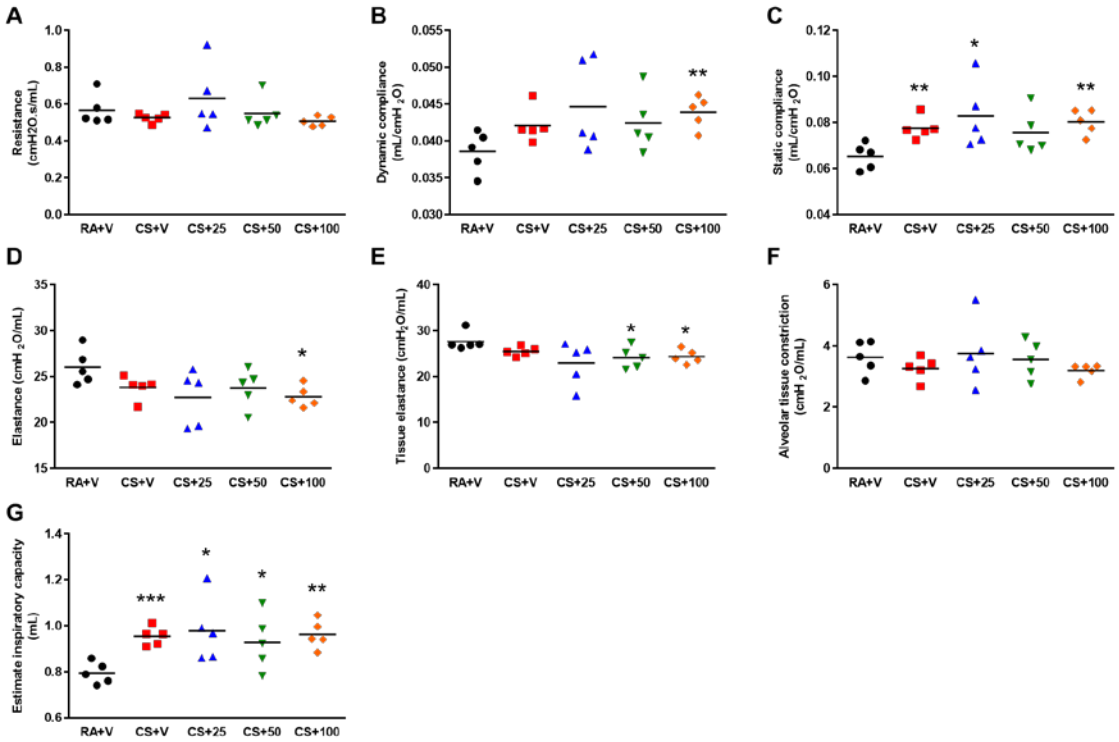

Figure 6. The effect of MIF20 on pulmonary function in short-term cigarette smoke (CS)-exposed male mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). Mice were orally treated vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. After anesthesia and paralysis, the mice were subjected to the following lung measurements, (A) resistance, (B) dynamic compliance, (C) static compliance, (D) elastance, (E) tissue elastance, (F) alveolar tissue constriction, and (G) estimate inspiratory capacity were measured by FlexiVent (Scireq, Montreal, Canada). The data are shown as mean ± SEM (n=5 per group). *p<0.05, **p<0.01, ***p<0.001 vs. RA+V group. RA=room-air.

Analysis/Interpretation: CS induced increased static compliance and estimated inspiratory capacity in female mice, indicating the presence of early emphysema and validating the effectiveness of our CS exposure system. However, MIF20 did not improve these parameters, suggesting that there is a differential effect of MIF20 on female vs male mice during short-term CS, where females may benefit more than males.

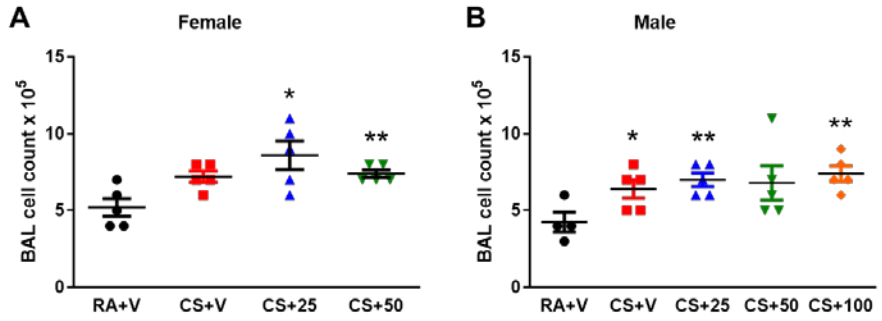

Figure 7. The effect of MIF20 on bronchoalveolar lavage (BAL) cell number in short-term cigarette smoke (CS)-exposed mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). Mice were orally treated vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. After anesthesia and paralysis, mouse trachea was cannulated and perfused with two 0.9 mL aliquots of cold-PBS. The cellular contents and BAL fluid were separated by centrifugation. Cells were re-suspended in PBS and cell counts were obtained via a Coulter counter (Beckman Coulter, Brea, CA). The data are shown as mean ± SEM (n=5 per group). *p<0.05, **p<0.01 vs. RA+V. RA=room-air.

Analysis/Interpretation: One of the major characteristics of COPD is inflammatory changes and cellular recruitment. We measured the cell number in BAL (broncho-alveolar lavage) fluid isolated from the lungs of mice. In female mice, there was tendency of increased BAL cell numbers, suggesting cellular inflammation, in the CS-exposed group, and MIF20 treatment augmented this increase. In male mice, CS increased BAL cell numbers as well but MIF20 did not affect this increase. This suggests that MIF20 again has a differential effect in female vs male mice and that MIF20 is recruiting cells to the lungs. Next, we will determine the cell subpopulations (neutrophils, macrophage, etc…) in female vs mice. MIF signaling pathway is necessary to maintain cell homeostasis and proliferation, but its pro-inflammatory and/or macrophage-recruitment properties may be responsible for the cellular recruitment. However, the cellular recruitment may include reparative, rather than deleterious, cell populations. The conclusion of the long-term CS will clarify whether the short-term CS BAL cell counts after MIF20 is reparative or deleterious. As an alternative, we can test the related MIF protein, d-dopachrome tautomerase (d-DT; MIF2) in CS-induced and/or *S. pneumonia*-induced lung injury. MIF2 is related to MIF protein but lacks the protein domain mediating inflammatory and cellular recruitment effects, which may mitigate the potential pro-inflammatory effects of MIF20.

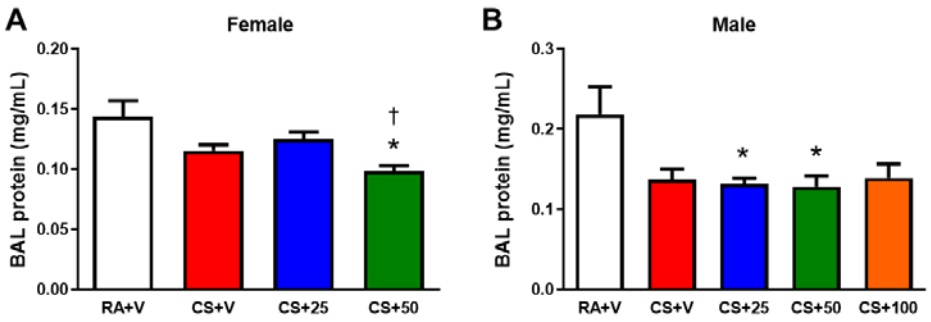

Figure 8. The effect of MIF20 on bronchoalveolar lavage (BAL) protein amount in short-term cigarette smoke (CS)-exposed mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). Mice were orally treated vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. After anesthesia and paralysis, mouse trachea was cannulated and perfused with two 0.9 mL aliquots of cold PBS. The cellular contents and BAL fluid were separated by centrifugation. Protein amount was measured by BSA reagent in BAL fluid (Thermo Scientific, Waltham, MA). The data are shown as mean ± SEM (n=5 per group). *p<0.05 vs. RA+V group. †p<0.05 vs. CS+V group. RA=room-air

Analysis/Interpretation: Nagai et al (Thorax 2006; 61: 496) reported that the total protein in BAL fluid from young smokers was lower than those from young non-smokers in human. Similarly, in our studies both female and male mice exposed to short-terms CS exhibited decreased total protein content in BAL fluid. MIF20 prevented the decreased protein content in female mice, but not male mice. We will measure the production of specific proteins in BAL, such as inflammatory cytokines, chemokines, and senescence-associated secretory proteins, to determine which proteins are altered by CS and MIF20.

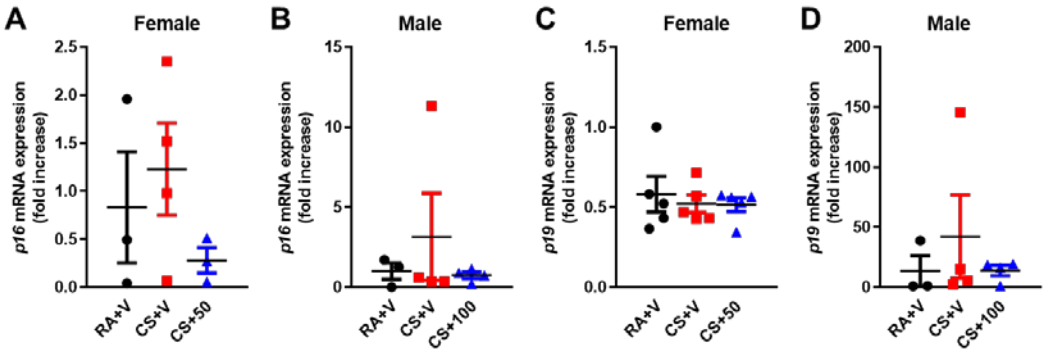

Figure 9. The effect of MIF20 on senescence-related gene expression in short-term cigarette smoke (CS)-exposed mice. The mice were exposed to CS in Teague machine for 3 months (6 h/day, 5 days/week). Mice were orally treated vehicle (V), 25, 50 or 100 mg/kg of MIF20 during the last 1 month. After anesthesia and paralysis, mouse lung tissues were collected and measured (A and B) *p16* and (C and D) *p19* mRNA expressions using real-time qRT-PCR method. The gene expressions were normalized by that of *gapdh*. There was no differences between groups in *p16* and *p19* mRNA expressions. The data are shown as mean ± SEM (n=5 per group). RA=room-air.

Analysis/Interpretation: The contribution of CS and aging to the pathogenesis of COPD is clinically known but the cellular and molecular underpinnings remain limited. Cellular senescence, a process that imposes permanent proliferative arrest on the cells, has emerged as a potentially important contributor to age-related disease. A few studies demonstrated the presence of molecular markers of senescence in the lungs of people with emphysema, an important feature of COPD. To determine the effect of CS and MIF on cellular senescence, we measured *p16* and *p19* mRNA expression. We did not detect significant differences between the groups, but the time point may not have been appropriate. Changes in senescence gene markers, such as *p16* and *p19* mRNA, can be variable over a lifetime of the mouse or during chronic environmental exposures, such as CS. Because the age of sacrificed mice was 5 months, it might be not enough to show the changes in senescence markers. Thus, we will measure the *p16*, *p19*, *p21*, and *p53* mRNA expression in 6 months CS-exposed mice (the age of sacrificed mice is 8 months) as well as earlier time points.

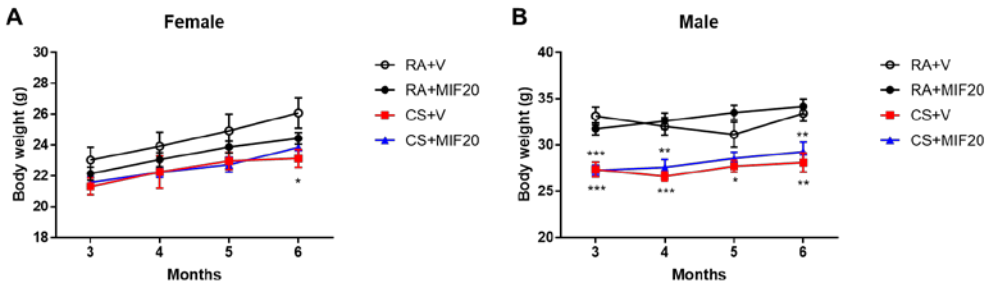

Figure 10. The effect of long-term cigarette smoke (CS) exposure and MIF20 treatment on body weight. The mice were exposed to CS in Teague machine for 6 months (6 h/day, 5 days/week), and were orally administered vehicle (V) or 100 mg/kg MIF20 during the last 3 months. The body weight of (A) female and (B) male mice were measured during MIF20 treatment. After 6 months CS exposure, CS groups showed decreased body weight in both female and male mice. The data are shown as mean ± SEM (n=7 for RA+V and RA+MIF20 groups, n=8 for CS+V and CS+MIF20 groups; one mouse of CS+V group was dead during CS exposure). *p<0.05, **p<0.01, ***p<0.001 vs. RA+V group. RA=room-air.

Analysis/Interpretation: We followed the mice for near-moribund or body weight changes for 28 days during MIF20 treatment. During the 6 months CS exposure, 1 male mouse of CS+V group was dead. CS exposure induced body weight loss in both female and male mice. In female mice, MIF20-treated group showed a trend to recovery of body weight, whereas the male mice did not. This data suggests an interesting gender-related therapeutic effect of MIF20 during long-term CS, confirming our gender-specific findings during short-term CS.

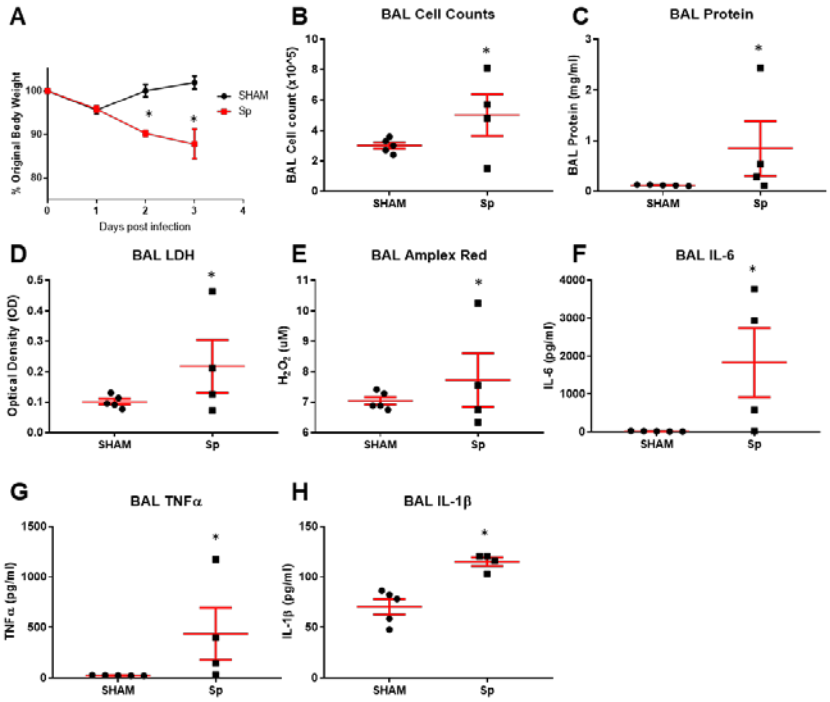

Figure 11. Effect of *S. pneumonia* injection on WT mice. (A) Body weights were measured at 1, 2 and 3 days post-infection. (B) Lung inflammation was detected by BAL cell counts. (C) Lung permeability was assessed by BAL protein content. (D) LDH activity assay from BAL fluid. (E) Oxidant generation was detected by Amplex red from BAL fluid. Cytokines were detected by ELISA in BAL as IL-6 (F), TNF-α (G) and IL-1β (H). The values are shown as mean ± SD. (n=5 for each group). *p <0.05 vs SHAM.

Analysis/Interpretation: Older adults and people with chronic lung disease, such as COPD, are particularly vulnerable to lung infections. *S. pneumoniae* is estimated to be a contributing organism in 20-60% of community-acquired pneumonia cases and is a leading cause of death among persons > 65 years old. In addition, CS exposure alone, in the absence of established lung disease, is a risk factor for the development of *S. pneumoniae* infections. To establish a clinically-relevant 2-hit lung injury model (CS + *S. pneumoniae* infection), we determined the effects of *S. pneumoniae* alone on body weight and inflammation. *S. pneumoniae* infection caused body weight loss and increased BAL cell number and protein amount, as well as LDH activity and oxidant generation (all measures of infection-related lung injury). In addition, *S. pneumoniae* infection induced inflammatory cytokine production in BAL fluid such as IL-6, TNF-α and IL-1β. There was no mortality during the experiments. Thus, we selected 10^5 CFU/mouse as the optimal concentration of *S. pneumoniae* for further study. We will test the optimal duration of CS following *S. pneumoniae* infection in the upcoming year and test the lung-effects of MIF20.

What opportunities for training and professional development have 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.

These studies resulted in new training opportunities by 1) promoting proficiency in medicinal chemistry and pharmacology, as necessitated by the manipulation of MIF20 in various diluents and concentrations; 2) proficiency in toxicity-testing, as necessitated by daily observations of mouse behavior, body weight measurements and lung histologic analyses; 3) proficiency in mouse handling and 4) proficiency in lung physiologic, cellular and molecular assays.
Professional development activities include broadening of scientific knowledge in lung biology, initiating new interactions with pharmaceutical colleagues (who provide MIF20) and the presentation of the results at Pulmonary research lab meetings and national and international conferences. In addition, opportunities to network with the Yale Office of Cooperative Research and biopharmaceuticals have formed as a result of these studies.

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.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

The next set of goals and milestones to achieve are the following:

1. Analyze cell-specific RNAseq data from 6 months CS-exposed mice.
2. Test the therapeutic efficacy of concurrent and post-treatment of MIF20 in the long-term (6 months) CS model.
3. Initiate the 2-hit model (*S. pneumoniae* injection following cigarette smoke exposure).
4. Test the therapeutic efficacy of MIF20 in CS-related *S. pneumoniae* infection.

4. **IMPACT:** Describe 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).

Our detailed testing of 3 doses of MIF20 and two different time-points for MIF20 treatment will expand our understanding of how MIF-based strategies may or may not be used to treat cigarette smoke-related lung diseases in both men and women. In addition, our short- and long-term cigarette smoke exposure models cover the major two characteristics of COPD—chronic inflammation and progressive destruction of lung structure.

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.

Our studies will impact the field of lung-targeted pharmacology and drug-development and, given that cigarette smoke negatively impacts most other organs, in addition to lung, such as brain, heart, eyes, pancreas, liver and gut, our studies on the potential therapeutic potential of MIF will have wide-ranging effects on many other disciplines. In addition, our studies will be one of the few of its kind to test MIF agonist strategies in a model of bacterial pneumonia after smoke-exposure.

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.*

We are performing fundamental *in vivo* testing of an immune modulator in a complex, chronic lung process for which no specific therapies exist and, therefore, these studies are critical to future drug-development for severe, chronic lung disease. These studies also allowed the initiation of a provisional patent and IP (intellectual property) filing through the Yale Office of Cooperative Research. MIF20 in COPD was selected as a semi-finalist for the Blavatnik Pitch Fest, occurring in Dec. 2018.

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.*

If these studies identify MIF as a therapy or as a diagnostic tool, the practice of medicine and delivery of treatment against cigarette smoke-related diseases as well as bacterial pneumonia would be significantly altered.

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:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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.

The original allocated salary for the operation of the smoking apparatus, Teague, had to be increased due to the level of training required. The original salary allocation was for the level of a postgraduate, but the complexity and technical skills required is at the level of a research associate (Emeka Ifedigbo, who has had >25 years of experience with mouse exposure systems).

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 or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

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).*

1. Wang C, de Mochel NSR, Christenson SA, et al. Expansion of hedgehog disrupts mesenchymal identity and induces emphysema phenotype. J Clin Invest. 2018; 128: 4343. doi:10.1172/JCI99435. PMID: 29999500.

2. Schwede M, Wilfong EM, Zemans RL, et al. Effects of bone marrow-derived mesenchymal stromal cells on gene expression in human alveolar type II cells exposed to TNF-α, IL-1β, and IFN-γ. Physiol Rep.2018;6(16):e13831. doi: 10.14814/phy2.13831. PMID: 30136410

3. Sauler M, Lamontagne M, Finnemore E, et al. The DNA repair transcriptome in severe COPD. Eur Respir J. 2018;52. pii: 1701994. doi: 10.1183/13993003.01994-2017. PMID: 30190272.

4. Zhang Y, Shan P and Lee PJ. Endothelial STC1 maintains mitochondrial bioenergetics and prevents oxidant-induced lung injury via TLR4. Antioxid Redox Signal. 2018. doi: 10.1089/ars.2018.7514. [Epub ahead of print]. PMID: 30187766

5. Sauler M, Bazan I and Lee PJ. Cell Death in the Lung: The Apoptosis--Necroptosis Axis. Annual Review of Physiology. In Press

6. Pellowe AS, Sauler M, Calderon B, et al. Endothelial cell secreted MIF regulates pericyte contractility during acute inflammation to decrease barrier function. FASEB J, In Press

7. Kim S, Shan P, Hwangbo C, et al. Endothelial Toll-like Receptor 4 maintains lung integrity via epigenetic suppression of p16INK4a. Aging Cell, Revision Submitted

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.*

American Thoracic Society 2018 International Conference (May, 2018, San Diego, CA)

- "MIF & Senescence"

Buck Institute of Aging, Astellas Pharmaceutical Board Members (January, 2018, Novato, CA)

- "MIF-COPD & Senescence"

Pulmonary Grand Rounds (March, 2018, Duke University, Durham, NC)

- "Vascular Basis of Lung Injury, Repair & Senescence"

Scientific Symposium, Pathobiology of Age-Related Lung Disease: From Bench to Bedside (May 2018, American Thoracic Society, San Diego, CA)

- "The Impact of Aging on Acute Lung Injury and ARDS"

Grand Rounds (September 2018, Emory University School of Medicine, Atlanta, GA)

- "Innate Immune Regulation of Lung Injury, Repair & Senescence"

Grand Rounds (September 2018, SFVA-UCSF Grand Rounds, San Francisco, CA)

- "Vascular Basis for Severe Lung Diseases"

9th International MIF Conference (October 2018, Munich, Germany)

- "Endothelial MIF as a Senescence-Associated Secretory Protein (SASP)"

Norway Aging Center Research Conference (October 2018, University of Norway, Akershus, NO)

- "Links between Innate Immunity & Senescence in Lung"

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.

Nothing to Report

Technologies or techniques

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

Nothing to Report

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.

Provisional patent and IP for MIF20 use in chronic lung diseases

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.*

Nothing to Report

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".

Name:	*Patty J. Lee, MD*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Lee supervised the overall design, experimental planning and data interpretation for all the studies.*
Funding Support:	*N/A*

Name: *Richard Bucala, MD, PhD*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *3*
Contribution to Project: *Dr. Bucala supervised the design and delivery methodology for MIF20.*
Funding Support: *N/A*

Name: *Heather G. Allore, PhD*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *2*
Contribution to Project: *Dr. Allore oversees all the biostatistical analyses and calculation for the bioassays and mouse outcomes.*
Funding Support: *N/A*

Name: *Taylor Ardito*
Project Role: *Postgraduate Associate*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *5*
Contribution to Project: *Ms. Ardito assists with mouse sample collections, lung function testing and maintains all human-related protocols for the Lee lab.*
Funding Support: *N/A*

Name: *Lin Leng*
Project Role: *Senior Research Scientist*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *6*
Contribution to Project: *Dr. Leng provides the MIF20 formulations and assisted with dose and delivery calculations*
Funding Support: *N/A*

Name: *So-Jin Kim*
Project Role: *Postdoctoral Associate*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *12*
Contribution to Project: *Dr. Kim worked in all MIF20 delivery mouse experiments, in addition to daily monitoring of the mice, mouse sacrificing and conducting lung assays.*
Funding Support: *N/A*

Name:	*Peiying Shan, MD*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Shan assisted in daily mouse monitoring/breeding, tissue processing and RNA sequencing of the mice.*
Funding Support:	*N/A*

Name:	*Emeka Ifedigbo*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Ifedigbo exposed mice to cigarette smoke and assisted Dr. Kim in MIF20 delivery and performing lung histologic analyses.*
Funding Support:	*N/A*

Name:	*Brent Vander Wyk*
Project Role:	*Biostatistician*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*1*
Contribution to Project:	*Mr. Vander Wyk works under the supervision of Dr. Heather Allore and assists with biostatistical analyses.*
Funding Support:	*N/A*

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.*

Nothing to Report

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.
Not applicable

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.