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Senescent Cell Evaluations in Normal Tissues (SCENT) Mapping Center Bronchoscopy Protocol

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We are still developing and optimizing this protocol

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Disclaimer

This is a Draft

Abstract

Cellular senescence is a stress-response, as well as a critical component of cell fate during development, repair, resilience, and normal aging. Deepening and broadening our investigations into cellular senescence in normal conditions will advance our knowledge of healthy aging as well as age-related disabilities, thereby leading to integrated and inclusive approaches to Gero-Protection and Gero-Therapeutics.

Goal and Objective

1 This project aims to collect, handle, store, and allocate normal healthy lung tissues and biofluids for constructing cellular senescence maps according to the standards established by the Steering Committee of the SenNet consortium. It seeks to identify senescent cell differences across the body, human health states, and lifespans. The NIH Cellular Senescence Network (SenNet) Program was established to comprehensively identify and characterize the differences in senescent cells across the body, across various states of human health, and across the lifespan. The Senescent Cell Evaluations in Normal Tissues (SCENT) Tissue Mapping Center (TMC) builds on the strengths at our institutions as well as external established research collaborations. As a centralized TMC for the SenNet consortium (Duke University – ISMMS), this study will provide a diverse, heterogenous cohort of respiratory track tissues samples from healthy individuals. To achieve this, a diverse, high-quality biorepository will be established through airway and blood sample collection from healthy adults. The goal is to provide detailed characterizations of cellular senescence in the lung and lung airway, and associated biofluids contributing significantly to advancing knowledge in human cellular senescence.

Study Procedures

2 Study Population/Setting
The study population will comprise healthy adult individuals from the Icahn School of Medicine and Mount Sinai hospital including Mount Sinai Hospital or ISMMS employees, students, volunteers, outpatients and any other members of the community. All research activities will take place at the Icahn School of Medicine at Mount Sinai in the Annenberg building 18th floor, room 96 (Dr. Patty J. Lee Lab) at 1468 Madison Ave, New York, NY 10029 and at the Bronchoscopy Suite also located in the 7th floor of the Annenberg building.

3 Recruitment Goal
Our goal is to enroll 40 healthy participants over the duration of this study. Our recruitment goal is highly feasible. We expect there will be large pool of eligible candidates since this project is targeting healthy adult individuals from the New York community.

4 Eligibility Criteria

4.1 Inclusion Criteria

- Healthy adult participants, ≥ 18 years old (Note: for this study “Healthy” is defined as participants who have no history of lung disease, allergies, and active respiratory infection symptoms)
- No significant active medical conditions as determined by the investigator
- Willing and able to provide informed consent and adhere to visit/protocol requirements.
- FEV1/FVC ratio > 0.70 and both FEV1 and FVC at least 80% of the predicted value (from spirometry)

- Normal Chest X-ray
- Normal CBC with differential, CMP, and INR (PT/PTT)

4.2 Exclusion Criteria

- History of asthma, chronic bronchitis, COPD, tuberculosis, hemoptysis, or recurrent pneumonia)
- History of respiratory/lung disease (e.g., COPD, asthma, emphysema, chronic bronchitis, tuberculosis, cystic fibrosis, lung cancer, recurrent pneumonia or other chronic lung disease)
- Active respiratory symptoms/infection within last 4 weeks
- Active smoking within the past year of conventional tobacco, inhaling of marijuana (smoking marijuana leaves or inhaling THC via e-cigarette) or other drugs. Vaping of e-cigarettes or vape pods >1 time per month in the past 6 months. Any form of tobacco qualifies, such as: 1 cigarette, 1 hookah or shisha sessions, 1 cigar, 1 pipe, etc. Any electronic (e)-device included: e-cigarette, mod, vape pen, JUUL, e-cigar, ehookah, e-pipe, vape pods, etc.
- Pregnancy or planned pregnancy in the next 2 months (i.e., during the duration of study procedures)
- Any prescription medication that while taking may be harmful to the participant, or detrimental to the research. All prescription medications will be reviewed by MPI to determine eligibility.
- Antibiotic administration or exacerbation/respiratory infection within the prior 30 days
- Underlying illnesses that may result in altered lung function (e.g., rheumatoid arthritis)
- Students or employees who are under direct supervision of any study member (to prevent undue influence or coercion)
- Allergies to medications used (or potentially used) in the study, including albuterol, acetaminophen, lidocaine, fentanyl, atropine, and midazolam.
- Any use of tricyclic antidepressants, beta-adrenergic blockers, aspirin, or other medications known to interfere with the treatment of anaphylaxis.
- Other medical or psychological conditions which, in the opinion of the investigator, might create undue risk to the participant or interfere with the participant's ability to comply with the protocol requirements.
- Nursing mothers
- Investigational medications within the last 30 days
- Poorly controlled concomitant conditions such as but not limited to obstructive sleep apnea, gastroesophageal reflux disease, chronic sinusitis/rhinitis, hypertension, diabetes where additional therapy/evaluation is required. The significance will be determined by the investigator
- Unwilling or unable to complete visit 1 procedures.
- Unwilling or unable to schedule or complete visit 2

5 Outreach

Outreach and recruitment efforts will occur through campus flyers, and social media. All public facing materials will be IRB approved prior to posting. The flyer will contain the study team contact information. The study team will contact the participant to assess eligibility. If eligible, the participant will be scheduled for visit 1.

6 Screening & Recruitment

Individuals interested in participating will reach out directly to the study team by email. The study team will then reach out to the interested individual to provide a description of the study, and to determine eligibility. If the individual meets the eligibility criteria, then they will be scheduled to come in for visit 1 (appendix 4). These individuals will be documented in the study EDC screening log. First and last name, date they reached out to the study team, their email, and phone number will be recorded in the EDC – screening log at this stage. If the study team is able to make contact with them, the screening questions (appendix 3) will be asked over the phone or a REDCap survey with the questions will be sent to them. All responses to the screening questions will be documented in the EDC. **Medical Record Number (MRN)**: if the participant is a new patient, they will receive a new MRN. This MRN will be recorded in the study EDC.

7 Study Visits

7.1 **Visit 1**: Visit 1 will occur in-person at a designated private space within the Annenberg building of the ISMMS campus. It is preferable that informed consent take place in person along with the subsequent study tasks, however remote consent (REDCap eConsent) will be used if necessary or if the study participant prefers.

Visit 1 consists of the following:

- Review of screening questions
- Informed consent
- Visit 1 Questionnaire (See appendix 4: Visit 1 Questionnaire)
- Pulmonary function test (PFT)/Spirometry pre and post albuterol
- Blood draw for CBC, PT/PTT and comprehensive metabolic panel

Informed Consent procedures: informed consent will occur at the beginning of visit 1, prior to any other study procedures. Consent can be done in person or remotely. The delegated study team member will summarize the entirety of the consent form and allow the participant to answer all questions throughout the entire process. The study team will ensure complete understanding of all study procedures and provide sufficient time for the participant to review and provide an informed consent. At no time will the study team use coercive tactics to obtain consent. The study team will obtain written informed consent. A REDCap e-consent will used primarily to record informed consent. However, if REDCap is unavailable for any reason, a paper consent will be used. If a paper consent is used, a copy will be uploaded to REDCap and the original will be housed in a locked cabinet in the Patty Lee lab at ISMMS.

If paper consent is used, the study team member will scan the signed consent and email the scanned copy to the participant. Additionally, if the participant wishes, a scanned physical copy will be provided to them in addition to an emailed copy (for documenting purposes).

7.2 **Visit 2**: Visit 2 will occur at the bronchoscopy suite in the Annenberg building at ISMMS. This visit will be scheduled if the individual completes Visit 1 and remains eligible to continue study participation.

Visit 2 consists of the following:

- Review of eligibility – i.e. reassess any changes to the screening questions.
- **Urine Collection**: used for pregnancy test (this will not be done for female participants older than 55)
- Spirometry pre and post albuterol
- Bronchoscopy procedure
- See 'Bronchoscopy Checklist' for additional details regarding visit 2
- Hospital procedure consent (separate from study consent) will be signed and dated on the day of procedure. The consent will be explained by the physician conducting the bronchoscopy.

Post-Visit 2 Procedures

8 Sample/Data Collection and Bronchoscopy

8.1 Visit 1

- Visit 1 Questionnaire (See appendix 4: Visit 1 Questionnaire)
- Spirometry pre and post albuterol
- Blood draw for CBC with differential, PT/PTT/INR and a comprehensive metabolic panel
- Chest radiograph if not performed in the last year

8.2 Visit 2

- **Nasal epithelial cells (right and left nares)** Nasal epithelial cells will be obtained by nasal brushings as described by Muller et al.1 Topical lidocaine will be used prior to the procedure to provide local anesthesia. Adequate cell numbers are obtained from this technique. With the participant sitting upright in a chair, nasal epithelial cells are obtained using a sterile cytologic brush. Briefly, brushings are obtained by gentle passage of the cuvette across the interior surface of the turbinate several times with a Rhino-Probe cuvette.
- **Bronchoscopy & related procedures** All eligible participants will undergo research bronchoscopy (performed according to NIH guidlines2) to obtain BAL fluid, endobronchial biopsies, and collection of airway cells from brushings of proximal epithelial. cells. Dr. Monica Kraft, a board certified pulmonologist with over 25 years experience in bronchoscopy including research bronchoscopy, will be performing the bronchoscopies. Other credentialed pulmonologists may be added to the protocol and will undergo IRB approval. Participants will then be prepared for bronchoscopy in the same manner as all patients and normal volunteers undergoing bronchoscopy at Mount Sinai. Medications used during the bronchoscopy procedure include lidocaine, midazolam, and fentanyl as part of conscious sedation.
- **Endobronchial Brushing**: A 3.0mm cytologic brush (ConMed, Utica, NY) will be introduced through the bronchoscope into the appropriate subsegmental bronchus and the bronchial wall will be brushed. For distal epithelial cell brushing, a cytologic brush will be placed through the left or right lower lobe bronchus and advanced to within 1-2 cm of the pleura as directed by fluoroscopy. Ten passes will be performed. Our experience using this technique for over 20 years yields approximately 5 million cells (approximately 95% epithelial). Cytospin preps will be prepared to evaluate the cell content. The purity of the brushed

epithelial cells will be verified by immunofluorescent staining using epithelial cell specific antibodies against keratin 19 (non-basal cells) and keratin 5 (basal cells).

- **Endobronchial Biopsy**: Endobronchial biopsies will be performed under direct visualization from the second to fourth generation airways. The right upper, right lower, left upper and left lower lobes are used with one sight used during each bronchoscopy. Four biopsies will be taken at the sub-carinae with an “alligator” type forceps.
- **Bronchoalveoloar lavage (BAL)**: The bronchoscope will be placed into the lung and the tip will be maintained in a wedged position. Five 60mL aliquots of 36°C sterile 0.9% saline solution are instilled after the bronchoscope is wedged into a subsegment. The bath water temperature is continuously measured. Samples of fluid will be removed through the bronchoscope by suction. The BAL fluid is harvested by immediate gentle hand suction applied to each instilling syringe. Syringes are placed immediately in ice. The volume of the effluent is measured after the procedure and samples are combined.3 Total cell counts, and cell differentials are done on the BAL by our standard method.4-6 Supplemental 02 will be administered as required. Medications before and during the bronchoscopy will be administered intravenously (IV). These medications are part of standard bronchoscopies procedures and as determined by the physician performing the procedure. Medications may include midazolam, fentanyl, and lidocaine to yield sedation, cough suppression and numbing of the throat and vocal cords.

9 Bronchoscopy Aftercare

The participant will be monitored in the recovery area of the bronchoscopy suite per clinical guidelines. The participant must have someone drive them home after this period of observation.

Additionally, the study team will contact the participant every-day for 3 days following the procedure to assess any adverse events, and one final call on day 14. The research team will ask the participant (preferably from the same household) for an additional contact person to call in the event the team is unable to get in contact with the participant.

10 Compensation

Participants will be compensated $40 upon completion of visit 1 and $460 upon completion of visit 2.

- Visit 1: In order to receive full compensation, individuals must (at minimum) complete and provide informed consent, complete visit 1 questionnaire, and perform the spirometry test. Partial compensation of $20 may be provided if some visit 1 procedures could not be completed due to unforeseen circumstances but this is up to the study team’s discretion. This will be explained to the individual during the consent process.
- Visit 2: In order to receive compensation for visit 2, the participant must complete the bronchoscopy procedure. However, in the event the participant arrives for visit 2 (i.e., the bronchoscopy) and the bronchoscopy team decide not to undertake the procedure due to safety concerns, then the participant will be partially compensated $40 for their time and travel expenses. In this situation, if visit 2 is rescheduled (assuming that the participant was partially compensated $40) then the participant will receive $420 for a total of $500 across both visits.

- If an unscheduled visit is required, the participant will receive an additional $50 in compensation

11 Study Duration

There is no definitive time frame for the overall duration of this study, however it is estimated that this study will last approximately 5 years.

There is no definitive time frame each participant's duration within the study. However, if the participant completes all study procedures, it is estimated that it will take approximately 2-4 weeks to complete both study visits. Visit 1 is approximately 1 hour, and visit 2 is approximately 6-7 hours.

12 Specimen Banking for Future Uses

All specimens will be banked for future use. Specimens will be stored in the Patty Lee lab located on the 18th floor, room 96 of the Annenberg building of the Icahn School of Medicine at Mount Sinai and will be store indefinitely. Investigators interested in accessing these specimens will need to provide a written request to the MPI. Additionally, investigators will either need to provide an approved IRB protocol detailing the use of these specimens or will be added to this study if the research is within the scope of this protocol. If external investigators wish to use these specimens, an approved IRB along with a fully executed MTA will need to be established prior to sharing any data or specimens. If the external investigator/institution is a member of the SenNet Consortium, this entity is covered under the MTA for the transfer of de-identified human tissues and specimens between non-profit organizations participating in the NIH Cellular Senescence Network (SenNet) program.

Under no circumstances will the study team share identifiable information. All data and specimens will be stripped of any 18 HIPAA identifiers prior to sharing. Only the study ID (e.g., 001, 002, 003, etc.) will be shared. The study team link identifying study participants will be retained by the study team.

13 Assays

Several assays will be performed. Immunohistochemical analyses will be performed using unique and validated marker panels in either lung or colon-derived specimens. Xenium technology, a new-generation spatial transcriptomic identification, will provide increased spatial resolution across lung and colon tissue. Along with pre-designed Xenium panels (Lung and Colon), senescence-specific custom gene panels will be created for use in the Xenium platforms. SnRNAseq and snATACseq analyses will also be performed on lung samples and colon specimens. We will use multiplex ELISA to determine senescence-associated secretory phenotypes (SASPs) on biofluids. Epigenetics (Methyl array) analyses will be performed to identify the DNA methylation patterns that distinguish senescence from normal cells and tissues. Additionally, Olink HT (high throughput) will be performed for unbiased, high throughput screening of proteins in BALF and plasma or serum. Core laboratory services will be utilized for several assays such as Olink, snRNAseq and snATACseq. No PHI, or PII will be handled by core services. All samples and data will be coded with a unique identifier. A coded link will be maintained to the data; however, this link will be retained by the study team.

14 Participant Study Status

It is important to note that for this study, there are several different types of statuses a participant can have. Study statuses are dynamic and change depending on where the individual is within their study participation.

14.1 **Consented**: defined as any individual who is approached for consent and voluntarily chooses to consent to the study, the procedures, risks and benefits with the understanding that they may withdraw at any time. A signed/dated informed consent must be on record to account for this individual. Individuals who are assigned this status must be noted on the enrollment log.

14.2 **Declined:** defined as any individual who was approached for consent but opted to NOT consent and therefore to NOT participate in the study. Individuals who are assigned this status must be noted on the screening log.

- Note: a “decline” may also be defined as an individual who was eligible at pre-consent but never followed through on a consent visit or an individual who opted to undergo a consent visit but deferred consent and the study team was never able to obtain a definitive “yes” or “no” after 3-5 attempted contacts.

14.3 **Enrolled:** defined as any patient who was consented, completes at visit 1 and scheduled for visit 2, has not been withdrawn, or has not screen failed. Participants who require visit 2 rescheduling will be still be considered enrolled.

14.4 **Screen Failed:** defined as any participant who consents to the study, completes visit 1 but does not meet continued eligibility criteria. This is to be documented on the enrollment log.

14.5 **Withdrawn by PI or Self**: defined as any individual who voluntarily consents to participate in the study, but is then withdrawn from the study.

- The PI may withdraw a participant at any time without their consent if the PI deems it is in their best interest to no longer participate OR the participant demonstrates a continuous lack of willingness or compliance with study protocol and study procedures.
- The participant may withdraw themselves at any time; the study team will be asked to document the reason for withdraw for study records.
- Participants who reach this status will have no additional follow-ups or study requirements. Participants who are assigned this status must be maintained on the enrollment log. A reason for withdraw should be recorded in the study records.

14.6 **Completed**: defined as any individual who consented to the study and completed visit 2. Individuals who reach this status have no additional study activities or study requirements. However, participants are encouraged to reach out to the study team if they experience any adverse events following any study procedures.

15 Statistical Design

This study design does not involve hypothesis testing and is observational in nature and therefore there is currently no statistical plan. Any planned research that involves the specimens and data collected under this protocol, and that is outside of the scope of this research will be submitted to the IRB for approval which may require either amending this

protocol or the submission of a separate protocol. The study team will seek the guidance of the IRB should this occur.

Investigators and collaborators seeking to obtain specimens and data from this biorepository must receive IRB approval for their respective study which may necessitate statistical considerations for their respective projects.

16 Data Management

- **REDCap**: as part of this project, the secure, electronic data capture (EDC) known as REDCap will be utilized for the purposes of maintaining a robust screening and enrollment log and housing, at a minimum, and the study eConsent. For data validation, a series of project-defined data checks and conditional constraints will be performed to ensure the highest quality data collection. The study team will use login/password credentialing for authentication. User-level permissions will be based on user roles and defined within the project system to limit a user's access to only those records an individual is authorized to see. Data will never be shared outside the project unless authorized by the MPI, and IRB compliant, in addition to a fully executed MTA or DTA, whenever necessary.
- **Sharepoint:** This will be utilized for the purposes of maintaining a robust screening and enrollment log, if necessary. The primary screening and enrollment log will be managed on REDCap. For data validation, a series of project-defined data checks and conditional constraints will be performed to ensure the highest quality data collection. The study team will use login/password credentialing for authentication. User-level permissions will be based on user roles and defined within the project system to limit a user's access to only those records an individual is authorized to see. Data will never be shared outside the project unless authorized by the MPI, and IRB compliant, in addition to a fully executed MTA or DTA, whenever necessary.
- **Data Quality control**: the delegated project manager will periodically (at minimum once per month) review the database, and sample inventory to assess completeness, timeliness, and accuracy. This plan will ensure the reliability of the data and will serve to make improvements, if necessary, to the study electronic database, procedures, workflow, and/or study protocol.
- **Specimen Quality control**: the delegated project manager will periodically (at minimum once per month) review procedures regarding the collection, transport, processing, and storage of all biological specimens and fluids. This will entail reviewing the sample inventory for completeness (cross-referenced with the associate participant's study progress) to ensure the completeness of data input.

Saftey

17 Protection Against Risks

Unauthorized Access to Data: There is a possibility of an unauthorized party gaining access to secure PHI contained in the database. This is also a low risk, and several precautions have been implemented to minimize unauthorized access. First, reports from participants' records concerning research observations will not be made available to outside medical facilities

without the written consent of the participant. All data obtained from research interviews and the laboratory will be coded. The data will be secured and accessible only to authorized research personnel. Only study numbers will appear on specimens, data and documents used for evaluation or statistical analysis. In addition, any publications resulting from this research will not identify individual participants.

Risk of Chest X-ray: This involves a small amount of radiation. The chest x-ray will be carried out on one occasion. The above radiation exposure is not necessary for medical care and is for research purposes only. This use involves minimal risk and is necessary to obtain the research information desired.
To put your estimated effective dose in perspective, the radiation that you will get for this research study will be less than the average person in the United States receives each year from natural sources (sun, outer space, air, food, and soil) and medical procedures. Based on these calculations, the risk from the radiation exposure in this research study is very small
Risk of Blood Draw: Risks associated with drawing blood include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, dizziness or fainting are also possible, although unlikely.
Risks/discomforts to questionnaires: While there are no apparent risks from the questionnaires some questions may be uncomfortable for some individuals. Any questions that are uncomfortable to answer may be skipped. However, if the questions being skipped are used to determine eligibility, then the individual will not be allowed to participate in the study due to safety concerns.
Risk of Intravenous Catheter: Insertion of the intravenous catheter (IV needle) into the arm involves the same risks as drawing blood, but the participant may also experience discomfort from having the catheter taped to their arm. In about 10% of cases, a small amount of bleeding under the skin will produce a bruise (hematoma). The risk of temporary clotting of the vein (from the IV) is about 1%, while the risk of infection of a hematoma or significant blood loss is less than 1 in 1000. Rarely there can be a severe infection of the blood stream or the heart valves or the formation of a blood clot that could go to the lungs. Complications are unlikely but treatment could require hospitalization. The IV catheter will be in place for approximately 4 hours.
Risk of Spirometry: The participant may become short of breath or experience dizziness due to requiring some exertion. To limit discomfort, the participant will be instructed to wear loose clothing and avoid eating for 12hrs before their scheduled visit.
Risks of Nasal Brushing: Risks associated with nasal brushing procedure include feeling a local burning with eye tearing, rarely local bleeding.
Risk of bronchoscopy: The participant may experience temporary coughing, vocal cord and bronchial spasms (airway narrowing), gagging, vomiting, fever, soreness of the throat or the procedure may involve a risk that is currently unforeseeable as a result of the bronchoscopy. More serious complications resulting in hospitalization or death can occur, but are extremely rare. There is also a risk of aspiration if the participant eats or drinks before the effects of the lidocaine have worn off. With the review of bronchoscopies performed, the overall rate of complications is 15% for any type of complication (including minor complications such as

coughing or sore throat) with a 5% risk for serious complications that may require further evaluation and medication. Appropriate treatment will be given if adverse events occur.
Risk of endobronchial biopsy: There is a risk of minor bleeding from the biopsy site. This may occur in up to 10% of the cases. The participant may expectorate blood-tinged sputum for 24 hours after the procedure as a result of minor trauma to the airway lining.
Risk of bronchoalveolar lavage: Pulmonary lavage may result in complications such as a fever, lung infection (pneumonia), a collapse of a small segment of the lungs at the lavage site (atelectasis), sharp chest pain and/or fever, vocal cord and bronchial spasms, and difficulty breathing. The study team follow up with the participant for several days after the procedure to monitor any complications that may arise. Should complications occur, the participant will be instructed visit the hospital and/or their primary care physician.
Risk of endobronchial brushing: There is a risk of minor bleeding from the brushing site. This may occur in up to 10% of the cases. The participant may cough up some blood for 24 hours after the procedure as a result of minor trauma to the airway lining which may occur. For the small airway brushings, the research team uses X-ray guidance which will expose the participant with a small amount of radiation. This risk is minimal and is necessary to obtain the research information desired.
Risk of sedation: The administration of a sedative medication may pose risks, such as problems with breathing, low blood pressure, heart rate, allergic reactions or, rarely, hospitalization or death. Also, sedation may cause temporary side effects including drowsiness, shaking, chills, dizziness, unsteadiness or forgetfulness. Appropriate monitoring equipment and medications will be used to promptly identify and manage complications if they occur. The participant will be instructed to not drive or make important decisions for 24 hours following the sedative medications. The participant will need to have someone escort them home. This will be explained to them prior to scheduling visit 2, the bronchoscopy procedure.
Risk of loss of private information: this risk always exists, but the study will implement strict study procedures to minimize this risk.
Group Risks: Although identifiable information is not shared to other investigators or entity, basic information such as race, ethnic group, and sex may be shared. This information helps investigators determine factors (if any) that lead to health problems between different groups of people. It is possible that such findings could be beneficial to certain groups of people. However, this could also be used to support harmful stereotypes or discrimination.
Risk of genetics: Because genetic information is unique to an individual, there is a small chance that it can be traced back to a specific person. The risk of this happening is very small but may grow in the future. Since the database will contain this genetic information, a break in security may also pose a potential risk to the participant's blood relatives. For example, it could be used to make it harder for you (or a relative) to get or keep a job or medical insurance. If this information is breached or misused, it is possible you would experience other harms, such as stress, anxiety, stigmatization, or embarrassment from revealing information about family relationships, ethnic heritage, or health conditions.

Confidentiality

Subjects will not be identified on any study reports. Mount Sinai firewalls, multiple passwords, and encryption programs protect the security of the electronic data entry system, which will be housed on a highly secure university server. All computers are located in lockable offices and

are accessible only by frequently changed passwords. Identifiable information that could directly identify a participant (based on any single 18 HIPAA identifier/ or any combination of information that could lead back to an individual) will only be accessed by the study team. All study members requiring access to identifiable information will undergo study onboarding training which will involve an overview of the entire protocol, and the standard operating procedures applicable to their role.

Privacy

The study team will closely safeguard participant privacy regarding protected health and personal information. A study ID number will be generated at the time of consent and will be maintained in a secure file (e.g., linker file) which will contain the participant's name and date of birth. Further telephone numbers, addresses, and other study related data will be stored securely and only accessible by delegated study team members.

Participant Death While in Study

While it is not anticipated that a participant will pass away given that this study is targeting healthy individuals, death may occur. If a participant passes away, information regarding their death will be collected by the study team to the best of their ability and immediately reported to the IRB as a serious adverse event. Information collected may include:

- Date of Death
- Cause of Death
- Location of Death

Withdraw While in Study

If an individual states that they wish to drop out, the study team will respect that as well. A follow-up question may be asked to ascertain the reason for withdrawing; however, this is for documentation purposes only and will serve to make any improvements, if necessary, to the study procedures. Additionally, the study and/or site PI retain the right to withdraw the participant from the study if they feel that participation is no longer in the participant's best interest and/or the participant is non-compliant despite numerous attempts to contact by the study team.

Vulnerable Populations

This project will not enroll individuals from vulnerable populations (e.g., imprisoned, pregnant, mental disabilities); however, it will recruit Mount Sinai Employees and students (except for employees and students under the direct supervision of the study team)

18 Provisions to Protect the Privacy Interests of Subjects:

Protecting participants' privacy in research is crucial for their comfort, respect, and confidentiality. The guidelines include obtaining informed consent by clearly explaining research objectives and potential risks, ensuring anonymity and confidentiality through coded data and secure storage, minimizing data collection, and securing data handling with encryption. Access to participants' data is restricted to specific team members with role-specific access determined by the principal investigator. Secure communication methods are

used, especially for sensitive topics, with an option for private responses (secured emails or direct conversations with the study team). Participants are informed of their right to withdraw without penalty. To make participants feel at ease, researchers build trust through transparency and empathy, communicate clearly, minimize intrusiveness, provide support, and design questionnaires with privacy in mind. Overall, approaching participants ethically and respecting privacy interests are essential for ethical and effective research.

19 Adverse events (AEs), serious adverse events, (SAEs) and unanticipated problems (UPs):
It is recognized that there is a slight risk that some participants may become distressed when completing self-report questions or participating in the study procedures, however this is not expected. All participants will have access to the PI's contact information should they have any questions (shown in the consent form).

It is anticipated, in this study, for AEs to be rare. However, it is possible that participants could experience situations outlined in the 'Risks' section of this protocol. Like, AEs, it is not anticipated that SAEs will occur. However, for this study, an SAE would be defined as a hospitalization, or death. All serious adverse events will be reported within the standard timelines as appropriate and when applicable.

Protocol deviations or unanticipated problems occur, they will be discussed with the PI, documented, and reported as appropriate and when applicable.

20 Period and Frequency for Event Assessment and Follow-Up
Protocol deviations and other unanticipated problems, as well as AEs and SAEs, will be recorded throughout the study and reported, as appropriate, and when applicable.

The study team will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. Events will be followed for outcome information until resolution or stabilization, or participation has ended.

21 Characteristics of an Adverse or Serious Adverse Event

21.1 Relationship to Study Intervention
To assess relationship of an event to this study, the following guidelines are used:

1. **Related (Possible, Probable, Definite)**

- The event is known to occur with the study procedures.
- There is a temporal relationship between the procedure and event onset.
- The event abates when the procedure is discontinued.
- The event reappears upon a re-challenge with the procedure.

1. **Not Related (Unlikely, Not Related)**

- There is no temporal relationship between the procedure and event onset.
- An alternate etiology has been established.

22 Expectedness

The PI will be responsible for determining whether an event is expected or unexpected. An event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention.

23 Severity

The following scale will be used to grade adverse events:

1. Mild: no intervention required; no impact on activities of daily living (ADL)
2. Moderate: minimal, local, or non-invasive intervention indicated; moderate impact on ADL
3. Severe: significant symptoms requiring invasive intervention; subject requires medical attention, needs major assistance with ADL

24 Reporting Procedures

Adverse and Serious Adverse Events

All adverse and serious adverse events will be reported to the IRB, and the study sponsor in the timelines provided by institutional and federal guidelines if deemed reportable by the PI.

Protocol Deviations and Other Unanticipated Problem Reporting

Incidents or events that meet the reporting criteria, as outlined by the Duke and Mount Sinai IRBs (web links provided below), will be reported.

Duke University:
https://irb.duhs.duke.edu/reporting-irb

ISMMS:
https://icahn.mssm.edu/files/ISMMS/Assets/Research/PPHS/ISMMS%20Instructions%20for%20RNI%20Reporting%20(04.21.2022).pdf

The following will be included, at a minimum:

- A detailed description of the event, incident, experience, or outcome.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

25 Monitoring

Data and Safety Monitoring Plan

The PI will monitor for, review, and when necessary, will promptly report to the IRB, institutional officials, sponsor, and any other entity as advised by the IRB - unanticipated problems involving risks to subjects or others that occur in the course of a subject's participation in research, all AEs and SAEs reports will be reported per the Duke and Mount Sinai IRB policies.

The MPI and the delegated project manager will supervise the study, including data management, data accuracy, and protocol compliance. The project manager will be the chief data manager and will adhere to established federal and institutional research participant safety and protection guidelines. To assure data accuracy, the project manager will review data

system reports on a routine basis. These reports will show enrollment, missing data, and other pertinent values and will be presented and evaluated along with the PI at study team meetings. Additionally, the project manager will process detailed reports to search for errors and generate basic reports for dissemination for regular staff meetings.

26 Vulnerable Populations

In addressing potential vulnerabilities, the study team acknowledges and endeavors to counteract power imbalances within historically marginalized communities. Mitigating coercion risks involves providing fair compensation and maintaining transparent communication, especially for individuals facing economic challenges. To enhance comprehension, the study team advocates for simplified consent forms and the use of alternative communication methods, such as visual aids, particularly for those with lower education levels. Safeguarding the rights of individuals with uncertain legal status includes ensuring confidentiality and clearly communicating the voluntary nature of participation. Extra precautions are necessary when involving cognitively impaired minors, necessitating assent and the involvement of legally authorized representatives. Additionally, safeguards should be in place for individuals in subordinate roles to ensure voluntary participation, confidentiality, and accessible avenues for raising concerns. To ensure diversity, the study team utilizes various recruitment channels, culturally sensitive materials, engages with community organizations, provides language accessibility, and continually monitors demographics for adjustments throughout the study.

27 Ethics

Duke University Federal Wide Assurance (FWA#00009025)
Icahn School of Medicine at Mount Sinai Federal Wide Assurance (FWA#00005656, FWA#00005651)
Information regarding registration of IORGs and IRBs, and the FWA status of an institution is available online: Click here to access the online database at **http://ohrp.cit.nih.gov/search/**

Appendix

28 Appendix 1: Abbreviations and Definitions

- **Adverse Event (AE):** Any untoward medical occurrence associated with or observed in the context of a study procedure. For this study and patient population, an AE will be considered any suicidal ideation. No other events will be considered AEs as this patient population is not ill and it is expected that other untoward medical occurrences will occur.
- **Cellular Senescence Network (SenNet):** Program was established to comprehensively identify and characterize the differences in senescent cells across the body, across various states of human health, and across the lifespan. SenNet will provide publicly accessible atlases of senescent cells, the differences among them, and the molecules they secrete, using data collected from multiple human and model organism tissues.
- **Electronic Data Capture (EDC):** For this study, the EDC will be supported by REDCap in which relevant study data, such as screening, enrollment, and clinical variables both from research participants' medical records and app will be documented.

- **Healthy Adult**: Individual who is at least 18 years of age, self-report no chronic lung disease (e.g., COPD, emphysema, cystic fibrosis, lung cancer or any other type that the PI deems not suitable for this study)
- I**cahn School of Medicine at Mount Sinai (ISMMS)**
- **Institutional Review Board (IRB)**: Ethical and regulatory committee who provides approval and oversight of clinical trial at study site.
- **Material Transfer Agreement (MTA)**: a contract that governs the transfer of tangible research materials between two organizations, when the recipient intends to use it for his or her own research purposes.
- **Mount Sinai Innovation Partners (MSIP):** facilitates the execution of DTAs, DUAs, MTAs
- **Multiple Principal Investigators (MPI):** PI's include Patty Lee, and Monica Kraft from ISMMS and Andrew Nixon from Duke University.
- **Protocol Deviation (PD):** An inadvertent event or event that is out of the control of the study team and/or the subject that occurs outside of the study protocol design and/or procedures.
- **Protocol Violation:** An act of intentionality that is committed by the study team and/or the subject that occurs outside of the study protocol design and/or procedures.
- **Screen fail:** Attains an FEV1/FVC ratio ≤ 0.70, and both FEV1 and FVC ≤ 80% predicted value from the spirometry assessment. Additionally, if there are any changes to the screening questions in visit 1. These participants will not be scheduled for Visit 2.
- **Serious Adverse Event (SAE):** Defined as an adverse event that is both serious and expected in nature; the event may have a reasonable possibility that it is related to a study. SAEs for this study are defined as a hospitalization, or death.
- **Tissue Mapping Center (TMC)**
- **Unanticipated Problem (UP):** Any other event, not meeting the definition of PD, UP, AE or SAE that, in the opinion of the principal investigator, merits documentation as it occurred outside the expected design of the study and/or study procedures. These events, like PDs, UPs, AEs, or SAEs, may be reported to the IRB and/or the study sponsor, as applicable.

29 Appendix 2

	A	B	C	D
	Sample Tissue Type	Study Visit in which collection occurs		Approximate Quantities (if applicable)
		Visit 1	Visit 2	
	Bronchoalveolar lavage (BAL)		X	60cc (amount will vary based on subject and or procedure)
	Bronchial biopsy		X	1 biopsy
	Left nasal brushing		X	1-2 million cells

	A	B	C	D
	Right nasal brushing		X	1-2 million cells
	Airway epithelial cells (brushing)		X	1-2 million cells
	Blood	x		Up to 5 tablespoons (approx. 74cc)

30 Appendix 3: Screening questions

- Are you at least 18 years old?
- Do you have a history of respiratory/lung disease (e.g., COPD, asthma, emphysema, chronic bronchitis, tuberculosis, hemoptysis, cystic fibrosis, lung cancer, recurrent pneumonia or other)

  If response is 'other' or 'not sure' – please describe (MPI will determine eligibly based on response of this question)

  If yes – participant is not eligible.

  If no – continue.
- Are you current pregnant, or plan to be pregnant during the duration of this research?
- Are you currently taking prescription medications?

  If yes, please describe (MPI will determine eligibility based on this response)
- Non/never smoker or former smoker (if they have not smoked (former) within the past 5 years and no history of 5 or more pack years) – smoking (inhaling of any smoke) in any capacity (i.e, traditional cigarettes/tobacco, cigars, marijuana, vaping, e-cigarettes)

  Smoking history

  Are you currently a smoker?

  If, not have you ever smoked in the past but quit?

  (if former) How long ago did you quit smoking?

  (If current, or former smoker)

  What types(s) of smoking have you ever used? (check all that apply)

  Cigarettes

  Marijuana

  Vaping/E-cigarettes

  Traditional pipe or cigar

  Hookah or waterpipe

  Other (please specify):

  (if current, or former smoker)

  On average, how much did you smoke per day, and how for how long? (i.e. period of smoking)
- Are you willing and able to provide informed consent and adhere to visit/protocol requirements.
- Do you have history of allergies (e.g., food allergy, hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, COPD, tuberculosis, hemoptysis, or recurrent pneumonia)
- Are you allergic to any of the following?

albuterol, acetaminophen, lidocaine, fentanyl, atropine, and midazolam.

- Are you a nursing mother?
- Are you currently taking any medications (prescription or over-the-counter), or antibiotics?
  If yes, please provide name, dose, route of administration, and frequency.
- Do you have any other medical or psychological conditions that we have not discussed?
  If yes, please describe:
- Have you participated in research involving any investigational medications within the last 30 days?
- Do you have any of the following conditions? obstructive sleep apnea, gastroesophageal reflux disease, chronic sinusitis/rhinitis where additional therapy/evaluation is required?
- If yes, please describe:
- Do you have active respiratory symptoms/infection such as sneezing, runny nose, cough, fever, congestion?
- Have you had any respiratory symptoms/infection within the prior 30 days?
- Have you had a Chest X-ray within the last year?

Prompt: please request a copy of the Chest X-ray from your healthcare provider and bring it with you on visit 1. (if participant cannot retrieve a copy of the Chest X-ray, they will receive a new one as part of visit 1)

31 Appendix 4: Visit 1 Questionnaire Content

- First and Last name
- Date of birth
- Ethnicity
  Asian
  Black or African American
  White or Caucasian
  Hispanic or Latinx
  Native American or Alaska Native
  Native Hawaiian or Pacific Islander
  Middle eastern or North African (MENA)
  Other (please specify):
- Gender
  Male
  Female
  Non-binary
  Prefer not to say.
  Other (please specify):
- Have there been changes to any of the information you provided when we last spoke? (study team will review all questions with the participant to ensure eligibility)
  Changes, if any, will be documented in the EDC and eligibility will be re-assessed)
- List of current medications and any previous medications if taken within the past 1 month, including dose, frequency, and route of administration
- Occupation including time (in years) in that role.
- Current zip code and length of time (if current zip code is less than 6 months, former zip code will also be documented)

- Smoking history

Are you currently a smoker?

If, not have you ever smoked in the past but quit?

(if former) How long ago did you quit smoking?

(If current, or former smoker)

What types(s) of smoking have you ever used? (check all that apply)

Cigarettes

Marijuana

Vaping/E-cigarettes

Vape pods

Traditional pipe or cigar

Hookah or waterpipe

Other (please specify):

(if current, or former smoker)

On average, how much did you smoke per day, and how for how long? (i.e. period of smoking)

History of COVID-19

Have you ever experienced any symptoms that could be related to COVID-19? (e.g. fever, cough, shortness of breath, loss of taste or smell)?

Have you ever tested positive for COVID-19?

When were you tested?

Have you had COVID-19 more than once?

What is the approximate dates of each?

Please specify symptoms you experienced, in each occurrence

Did you experience any complications or long-term effects as a result of COVID-19?

If yes, please describe:

Did you need to seek medical care?

We're you admitted to the hospital as a consequence of this event?

32 Appendix 5: Schedule of Events

Appendix 5: Schedule of Events

	Screening	Visit 1	Visit 2	Post procedure Monitoring	Unscheduled Visit
+/-Window			Up to 4 weeks if continued to be eligible	Every day- first 3 days after Bronchoscopy	
ELIGIBILITY					
Informed Consent		X			
Visit 1 Questionnaire		x			
Inclusion/Exclusion	x	X	x		
Spirometry		X			
STUDY PROCEDURES					
Vital Signs		x	x		
Physical Exam		x			
Blood draw		x			
Urine pregnancy test (done in bronch suite)			X		
Chest X-ray		x			
Instructions for Bronchoscopy		x			
Bronchoscopy with biopsies, lavage and brushing			X		
Nasal epithelial brushing			X		
Increased Symptom management				x	X

33 Appendix 6: References

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6. Jagielo PJ, Thorne PS, Watt JL, Frees KL, Quinn TJ, Schwartz DA. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. Chest. 1996 Jul;110(1):263-70. doi: 10.1378/chest.110.1.263. PMID: 8681637.

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