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Title: Characterization of Tissue-Specific Immune Responses to Bronchoscopic Instillation of Mycobacterial Antigens into the Human Lung

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Drug Name:	Tuberculin Purified Protein Derivative
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Sponsor:	NHLBI OCD
Manufacturer:	Sanofi Pasteur

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

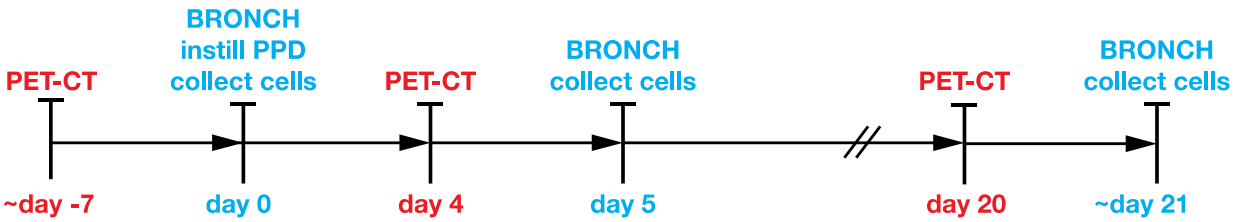
1.1 SYNOPSIS

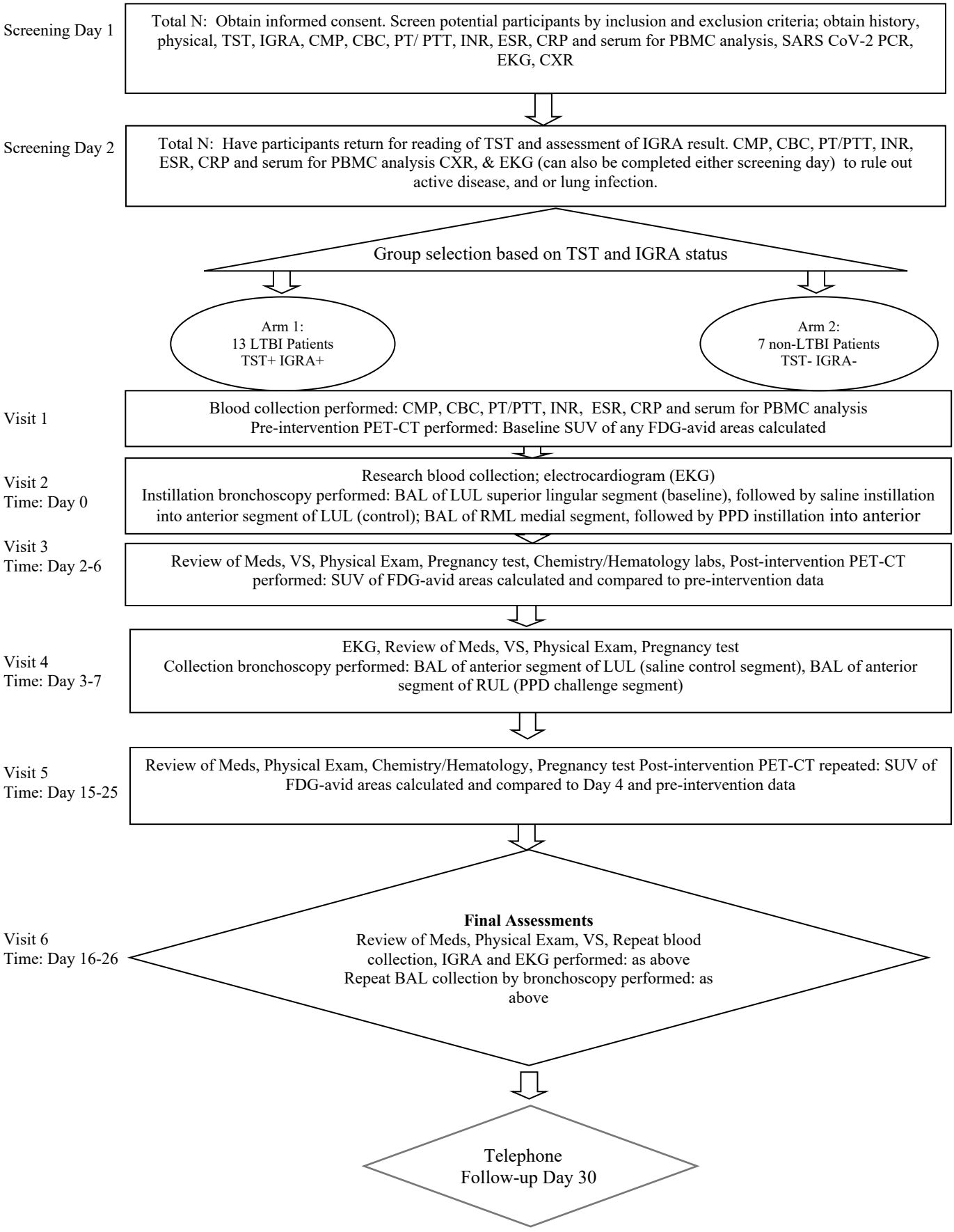
Title:	Characterization of Tissue-Specific Immune Responses to Bronchoscopic Instillation of Mycobacterial Antigens into the Human Lung
Study Description:	We propose a pilot study in which we employ directed bronchoscopic instillation of *Mycobacterium tuberculosis* (Mtb)- Tuberculin Purified Protein Derivative (PPD) to evaluate local airway immune response in adults with or without latent tuberculosis infection (LTBI).
Objectives	**Primary Objective:** The primary objective of this study is to understand the pulmonary immune response to mycobacterial antigens by determining the persistence of antigen-specific immune cells in the airways versus circulation after local bronchoscopic instillation of Tuberculin Purified Protein Derivative (PPD) into the lungs of adults with or without LTBI. **Secondary Objective:** The secondary objective is to characterize the location and persistence of immune cell activity in the pulmonary parenchyma and thoracic lymph nodes with positron emission tomography combined with chest computed tomography (PET-CT) after bronchoscopic Tuberculin Purified Protein Derivative (PPD) instillation in adults with or without LTBI. **Exploratory Objectives:** (a) An exploratory objective will be to perform phenotypic and functional analysis of immune cell populations in the airways vs circulation following bronchoscopic instillation of Tuberculin Purified Protein Derivative (PPD) into the lungs of individuals with or without LTBI. (b) An additional exploratory objective will be to characterize the production of soluble mediators (including but not limited to: cytokines, lipid mediators, and defensins) in BAL fluid vs blood within the same context.
Endpoints:	**Primary Endpoint:** The primary endpoint will be the enumeration of Mtb antigen-specific CD4+ and CD8+ T cell populations in the airway and peripheral blood at early and late time points after bronchoscopic Tuberculin Purified Protein Derivative (PPD)instillation. **Secondary Endpoint:** The secondary endpoint will be the quantification of [^{18}F]fluoro-D-glucose (FDG) uptake in adjacent pulmonary parenchyma and draining thoracic lymph nodes via PET-CT at early and late time points after PPD instillation. **Exploratory Endpoints:** (a) An exploratory endpoint will be the quantification of frequencies of cell surface molecules, intracellular cytokines and transcription factors among immune cell populations in the airways vs circulation following bronchoscopic instillation of Tuberculin Purified Protein Derivative (PPD) into the lungs of individuals with or without LTBI. (b) An exploratory endpoint will involve the identification and quantification of soluble mediators in the airway and peripheral blood after PPD instillation in the lungs within the same context.
Study Population:	Up to 100 male and female subjects will be screened in order to generate a Sample size: 13 confirmed latent tuberculosis infection (LTBI) cases and 7 confirmed non-LTBI controls Gender: male or female Age: 18 – 64 years of age Demographic group: no exclusions

	General health status: healthy volunteers (non-LTBI controls vs confirmed LTBI)
Phase:	Not applicable
Description of Sites/Facilities Enrolling Participants:	NIH Clinical Center: Procedures, Vascular Access and Conscious Sedation (PVCS) suite, Building 10; Radiology and Imaging Sciences Suite, Building 10; Laboratories of Daniel Barber and Katrin Mayer-Barber, Building 33
Description of Study Intervention:	Bronchoscopic instillation of 0.5 tuberculin units (10 microliters) of Tuberculin Purified Protein Derivative (PPD)(TUBERSOL®, Sanofi Pasteur Inc., Swiftwater, PA) diluted in 10 cc normal saline into the anterior segment of the right upper lobe.
Study Duration:	5 years
Participant Duration:	30 days

1.2 SCHEMA

General Schema Outline




Screening Day 1
Total N: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, physical, TST, IGRA, CMP, CBC, PT/ PTT, INR, ESR, CRP and serum for PBMC analysis, SARS CoV-2 PCR, EKG, CXR
Screening Day 2
Total N: Have participants return for reading of TST and assessment of IGRA result. CMP, CBC, PT/PTT, INR, ESR, CRP and serum for PBMC analysis CXR, & EKG (can also be completed either screening day) to rule out active disease, and or lung infection.
Group selection based on TST and IGRA status
Arm 1:
13 LTBI Patients
TST+ IGRA+
Arm 2:
7 non-LTBI Patients
TST- IGRA-
Visit 1
Blood collection performed: CMP, CBC, PT/PTT, INR, ESR, CRP and serum for PBMC analysis
Pre-intervention PET-CT performed: Baseline SUV of any FDG-avid areas calculated
Visit 2
Time: Day 0
Research blood collection; electrocardiogram (EKG)
Instillation bronchoscopy performed: BAL of LUL superior lingular segment (baseline), followed by saline instillation into anterior segment of LUL (control); BAL of RML medial segment, followed by PPD instillation into anterior
Visit 3
Time: Day 2-6
Review of Meds, VS, Physical Exam, Pregnancy test, Chemistry/Hematology labs, Post-intervention PET-CT performed: SUV of FDG-avid areas calculated and compared to pre-intervention data
Visit 4
Time: Day 3-7
EKG, Review of Meds, VS, Physical Exam, Pregnancy test
Collection bronchoscopy performed: BAL of anterior segment of LUL (saline control segment), BAL of anterior segment of RUL (PPD challenge segment)
Visit 5
Time: Day 15-25
Review of Meds, Physical Exam, Chemistry/Hematology, Pregnancy test Post-intervention PET-CT repeated: SUV of FDG-avid areas calculated and compared to Day 4 and pre-intervention data
Visit 6
Time: Day 16-26
Final Assessments
Review of Meds, Physical Exam, VS, Repeat blood collection, IGRA and EKG performed: as above
Repeat BAL collection by bronchoscopy performed: as above
Telephone
Follow-up Day 30

1.3 SCHEDULE OF ACTIVITIES (SOA)

Schedule of Activities (SOA)**EVALUATION AND ASSESSMENTS**	**Screen***	**Baseline**	**Study Visits 3 to 6**					**Telephone Follow- up Day 30**
PROTOCOL TIMEPOINT		**V1**	**V2**	**V3**	**V4****	**V5**	**V6****	
Window +/- days	**-60 Days**	**-7 Days**		**-2 /+3 D**		**-5 /+6D**		
day /month			**D0**	**D4**	**D5**	**D20**	**D21**	
Assessments								
Review Inclusion/Exclusion Criteria	**X**							
Consent to study	**X**							
Review Medical History with Mtb Exposure	**x[1]**							
Review of Medications	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Physical Examination	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Weight, Height, and BMI	**X**							
Vital Signs (BP, HR, Resp, Temp O_2 Saturation)	**X**	**X**	**X**		**X**		**X**	
Adverse Event Review		**X**	**X**	**X**	**X**	**X**	**X**	**X**
CHEMISTRY LABS								
Female, Pregnancy Test (blood or urine) [2]	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Acute Care Panel	**X**	**X**		**X**		**X**		
Mineral Panel	**X**	**X**		**X**		**X**		
CRP	**X**	**X**		**X**		**X**		
Hepatic Panel	**X**	**X**		**X**		**X**		
LDH	**X**	**X**		**X**		**X**		
International Normalized Ratio (INR), Prothrombin Time (PT), and Partial Thromboplastin Time PTT	**X[3]**	**X[3]**		**X[3]**		**X[3]**		
COVID-19 (PCR)	**X**							
HEMATOLOGY LABS								
CBC with differential	**X**	**X**		**X**		**X**		
ESR	**X**	**X**		**X**		**X**		
Viral Serologies								
Hepatitis B, C	**X**							
HIV	**X**							
Additional Tests								
***QuantiFERON Gold Test (IGRA)**	**X**						**X**	
***Tuberculin skin test (TST)**	**X[4]**							
EVALUATIONS								
EKG	**X**		**X**		**X**		**X**	
***Chest X-RAY**	**X**							
Chest PET-CT		**X**		**X**		**X**		
PROCEDURES								
Bronchoscopy			**X[3]**		**X[3]**		**X[3]**	

STUDY MEDICATION								
Installation of Tuberculin Purified Protein Derivative (PPD) and saline			**X**					
Removal of Tuberculin Purified Protein Derivative (PPD) and saline cells					**X**		**X**	
Research Samples								
Immunology Assays = 40 mL via heparinized green top tubes			**X**		**X**		**X**	

*** = Medical history screening activities may be done utilizing the Telehealth Visit Platform. Screening tests may be used as baseline if performed within 60 days. If re-screened after 60 days, the TST, IGRA, & chest x-ray will not be repeated unless there is a known tuberculosis exposure, including but not limited to travel to an endemic area or direct contact with an individual with known or suspected tuberculosis disease.**

x^1 = History of exposure to persons with active TB; History of residence in a TB-endemic country and Estimate of time when LTBI most likely occurred

x^2 = A plasma or urinary HCG in female subjects who are of child-bearing age will be collected within 7 days of study participation. While on study women of child-bearing age will have pregnancy test at each visit unless test has been performed within 24 hours of study visit

x^3 = Patient may not use platelet inhibitors (e.g. clopidogrel) within 7 days or systemic anticoagulants (e.g. warfarin, enoxaparin, or DOAC) within 14 days of bronchoscopy.

X^4 = Tuberculin skin test (TST) to placed prior to screening labs. A second TST may be completed per PI or designee if needed to confirm negative results one to three weeks after the first TST.

**** = Bronchoscopy for visit 4 and visit 6 will be performed within 2 days after the PET-CT**

2 INTRODUCTION

2.1 STUDY RATIONALE

Most of our understanding of human specific CD4 T cell responses to *Mycobacterium tuberculosis* (Mtb) is based on studies of circulating peripheral blood cells, and less is known about mycobacteria-specific CD4 T cells within the airways and alveoli. Recent studies in experimental mouse and macaque models by co-investigator Daniel Barber and colleagues have shown that this is a critical issue limiting our ability to understand protective cellular immune responses to Mtb (1-3). These functional differences between airway and blood T cells are poorly understood in humans within the context of tuberculosis infection (4). Furthermore, previous limited characterizations of the human airway immune cell phenotypes in the context of TB do not consistently correlate with mice and macaque models of Mtb pulmonary disease (5,6). For that reason, this proposal aims to address the gap in our knowledge of human Mtb airway immune responses not fully elucidated by animal models.

Additionally, the timing and location of early human immune responses to inhalation of *M. tuberculosis* are poorly understood. Data from animal studies have demonstrated that the mediastinal lymph nodes are the crucial primary site for the development of the adaptive antigen-specific CD4 T cell activation post aerosol exposure to Mtb (7,8). Additional work has demonstrated that intralymphatic administration of *Mycobacterium bovis*-derived vaccine provides improved protection from Mtb infection in mice as compared to intradermal and subcutaneous routes, reinforcing the importance of the thoracic lymphatic network for the establishment of protective immunity to Mtb (9).

However, there are few data in humans that shed light on the CD4 T cell amnestic response in the lung tissue vs draining mediastinal lymph nodes in the context of Mtb exposure. It is unclear whether this process is solely driven by antigen-specific T cells which mobilize to the site of inflammation over time or by a population of resident antigen-nonspecific effector T cells which are recruited to inflamed lung tissue based on a chemokine gradient. In a small pilot study of household contacts of patients with active pulmonary TB who were recently diagnosed with latent TB infection, we observed increased cellular activity of the thoracic lymph nodes using positron emission tomography combined with chest tomography (PET-CT), in spite of otherwise normal appearing lung parenchyma and lymph nodes on CT (10). ^{18}F-FDG PET-CT imaging is emerging as a very sensitive method for visualizing thoracic inflammation (11-13). For these reasons, longitudinal PET-CT images will provide critical non-invasive evaluations of cellular activity within the human lung parenchyma and draining mediastinal lymph nodes after exposure to Mtb antigen that provide insight into the location and timeline of anamnestic CD4 T cell immune response development.

2.2 BACKGROUND

Tuberculosis is caused by the inhalation of airborne Mtb bacilli. In animal models, alveolar macrophages are the first to engulf the bacilli, but eventually Mtb spreads to and grows within several other myeloid cell populations in the lungs (14,15). Approximately 1 to 2 weeks after initial infection, viable bacteria are transported via lymphatics to the draining hilar and mediastinal lymph nodes (16,17), and adaptive immune responses are then initiated (18). Recently, the use of PET-CT enabled us to demonstrate that there is early cellular activity in the

thoracic lymph nodes of recent household contacts of patients with active TB (10), but the early events of human Mtb infection remain poorly understood.

The treatment of active TB disease caused by drug-susceptible strains requires 4 antimycobacterial drugs for the first 2 months, followed by 4 months of 2 drugs. In the case of multidrug-resistant TB (MDR-TB), treatment requires more toxic drugs given for at least 18 months and usually for 24 months or longer. Extensively drug-resistant TB (XDR-TB) is often fatal in most of the world. In Cape Town, South Africa, where treatment is available, it is only successful in about 10-20% of cases (19). Thus, there is a pressing need for interventions to prevent infection or progression of disease. Vaccination with an attenuated strain of *M. bovis* BCG has been used for decades, but it is only useful in preventing disseminated disease, especially TB meningitis in infants. It does not prevent pulmonary TB, which accounts for over 85% of cases and is the form responsible for ongoing person-to-person airborne transmission.

Control of Mtb infection requires CD4 T cells, and if vaccination for Mtb is to be successful in the future, it will most likely be through the induction of CD4 T cell responses in the lungs. However, most of our understanding of human Mtb-specific CD4 T cell responses is based on studies of circulating peripheral blood cells, and little is known about mycobacteria-specific CD4 T cells in the airways. In animal models of tuberculosis, the Barber lab has shown that there are major differences between the T cells that enter the lungs vs those in circulation (1-3, 20). The primary purpose of this protocol is to study CD4 T cell responses in the human lung using bronchoscopic instillation of mycobacterial antigens in the form of tuberculin PPD, an established experimental approach that mimics exposure to inhaled Mtb. This method was first pioneered and proven safe by Silver and colleagues (21-23). Silver and colleagues have performed a limited immunological analysis on airway immune cells at day 2 post-instillation. Furthermore, this group routinely instilled PPD or control normal saline into the right middle lobe or lingula, the equivalent middle lung region on the left, where pulmonary TB rarely occurs. The major modifications of our approach is that we will perform a more comprehensive immunophenotyping of both peripheral and airway immune cells following PPD challenge at both early and late time points while evaluating local parenchymal and lymph node immune cell activity via PET-CT. Additionally, we will study PPD instillation and responses in the upper lobe of the lung, where most HIV-negative adults with pulmonary TB develop disease.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

Risks Related to Tuberculin Purified Protein Derivative (PPD):
PPD using the Mantoux method is the current standard for tuberculin skin tests (TSTs). Rarely, patients may develop a necrotic skin reaction at the site of intradermal injection. Screened subjects will be excluded from participating if they have a history of such skin reactions.
The model of direct lung segment challenge with PPD was established at the Case Western Reserve University School of Medicine as discussed above. It has subsequently been used in multiple subsequent studies by this research group (21-24). It results in a localized inflammatory response that is limited to the challenged sub-segment. None of these studies have reported serious or unexpected side effects with this model.

Risks Related to Blood Collection: Blood collection may cause some pain or bruising at the site on the arm from which the blood was drawn. There is a small possibility of fainting and infection. Similarly, there is a small risk of bleeding, blockage, or inflammation or infection of the vessel. Discomfort does not usually last long and permanent damage is extremely rare.

Risks Related to Bronchoalveolar Lavage (BAL): Subjects will sign a separate procedure consent for the BAL, which will further detail the potential risks of this procedure which include:

- Discomfort due to coughing. This can be controlled by topical medication.

- A decrease in the amount of oxygen in the blood. Subjects will receive additional oxygen during the procedure and they will be closely monitored for oxygen levels, heart rate and blood pressure. The risk of a serious problem occurring is very small (less than 1 out of 10,000 procedures in published studies).

- A vagal response may result in a transient slow heartbeat occurs (rare). These are self-limited and do not require medication. A heart rhythm monitor (electrocardiograph) will be used throughout the procedure.

- Mild bleeding from the nose can occur because the bronchoscope tube is sometimes placed through the nose to reach the large airways. Placing medication and lubricant inside the nose will be done before the procedure to lower the risk of this occurring. Alternatively, the bronchoscope can be introduced through the mouth.

- A sore throat may be present for several hours after the procedure (less than 10% of patients). This can be treated with topical anesthetics (e.g. throat lozenges)

- A reaction to the topical anesthetics (i.e. lidocaine, tetracaine or cetacaine) occurs that may include confusion or, very rarely, seizures. This potential problem is minimized by using small, frequent doses of the medication.

- Fever has been reported to develop in less than 5% of healthy volunteers six to eight hours after the bronchoscopy. In participants who develop a fever after bronchoscopy, the symptoms can be ameliorated with acetaminophen.

- Infection in the lungs resulting from aspirating gastric contents into the lung airways. To minimize this risk, subjects do not eat or drink for at least six hours before the bronchoscopy. There is also a risk of aspiration in the post procedure period during which a patient is recovering from effects of sedative administration. For this reason, each subject will be monitored closely within the procedural unit until the level of conscious has returned to baseline.

- With the use of conscious sedation there is a minor risk of change in baseline respiratory status (hypoxia, respiratory depression) or cardiovascular complications (hypotension, dysrhythmias). Midazolam and Fentanyl are the medications that will be used for conscious sedation. With the use of midazolam there is minor risk of vomiting and

decreased respiratory rate. With the use of fentanyl there is a minor risk of abdominal pain, constipation, nausea and vomiting. Each study subject will be informed of this risk and presented with the alternative of undergoing this procedure with the use of local anesthesia alone.
Risks Related to Chest PET-CT scans: Subjects will sign a separate procedure consent for PET CT scans, which will further detail the potential risks of this procedure which include:

- Radiation Risks: This study involves exposure to radiation from the Chest X-ray, and PET-CT scan and 18F radioisotope. At doses much higher than received in this study, radiation is known to increase the risk of developing cancer. Based on the doses of radiation planned for this study, the risk of inducing a neoplastic process is low. Each subject is planned to receive a total of 0.9 Roetgen equivalent man (REM) for each of the chest PET-CT Scan, thus receive a total of 2.7 REM during the study. Subjects will receive an additional 0.001 REM for the screening Chest X-ray, thus receive a total of 2.71 REM during the study. This is below the 5 REM annual dose limit recommended by the United States Nuclear Regulatory Commission (56 FR 23396). Each study participant will be informed of the magnitude of their radiation exposure and informed of their risk of exceeding the recommended annual dose limit if they receive additional significant radiation exposure outside of the study. A radiation exposure tracking tool, created by study personnel, will be completed to capture any radiation exposures in the prior 12 months at the time of enrollment and any additional outside exposures between study visits. This tracking tool may be completed via a Excel document and or utilizing radiation calculator tool via https://www.xrayrisk.com/calculator/calculator-normal-studies.php.

- Allergy Risk: There is a risk of allergic or other adverse type reaction to PET-CT radiotracers. This is extremely rare. Fluorodeoxyglucose (FDG) is a natural sugar that is attached to the radiotracer. There have not been any allergic reactions reported to FDG alone in the past. However, if a patient were to develop an allergic reaction, treatment would be initiated immediately with diphenhydramine, ranitidine, and/or corticosteroids depending on the severity of the reaction.

- Risk of Incidental Findings: Unanticipated clinically insignificant or potentially significant abnormalities may be detected from the results of the PET-CT scans performed. Such abnormalities due incur the risk of future potentially unnecessary additional diagnostic testing or therapeutic intervention, which can be associated with various complications.

- Reproductive Risks: Since PET-CT scans involve exposure to radiation which can lead to teratogenic effects to a fetus. For this reason, women of child-bearing age will undergo pregnancy testing prior to PET-CT scan and be excluded from the study if testing is found to be positive.

2.3.2 Known Potential Benefits

There are currently no known direct immediate benefits of this study for individual subjects. There is the potential benefit of identification and subsequent treatment of latent tuberculosis infection in a subject without prior knowledge of this infection prior to this study. However, this study has the major benefit that this study will provide important data relevant to the development of TB vaccines, diagnostics, and host directed therapies, as well as basic understanding of human lung immunity. For example, we expect to see different immune responses in those with LTBI versus those without. If this is confirmed, it would suggest that strategies for vaccination should differ for those with and without LTBI. Similarly, if we confirm that there is early cellular activity in the thoracic lymph nodes, it would be reasonable to develop drugs for early infection that target the thoracic lymph nodes, such as those in nanoparticles.

2.3.3 Assessment of Potential Risks and Benefits

Rationale for exposing study participants to the risk of diagnostic bronchoscopy and bronchoalveolar lavage: As stated above the functional differences between airway and blood T cells are poorly understood in humans within the context of tuberculosis infection. Additionally, limited evaluations of the human airway immune cell phenotypes in the context of TB do not consistently correlate with mice and macaque models of Mtb pulmonary disease. For these reasons, in order to obtain direct characterization of the human airway immune response to this pathogen transmitted via aerosol exposure we propose the aforementioned bronchoscopic instillation model. The risks of bronchoscopic instillation and bronchoalveolar lavage to participants will be minimized by the following means:

- Excluding participants with a past medical history or current medical issues which place them at risk of a decline in their baseline respiratory function during bronchoscopy
- Use of *M. tuberculosis* protein derivative instead of active or inactivated bacilli in order to make infectious risk to participant negligible
- Use of pulmonary arterial catheter for direct bronchial challenge, limiting lobar dissemination of PPD
- Absence of performance of bronchial or pulmonary parenchymal biopsy sampling to limit the risk of procedure-related pneumothorax
- Excluding current use or inability to suspend use of anticoagulant including platelet inhibitors (e.g. clopidogrel) within 7 days of bronchoscopy or systemic anticoagulants (e.g. warfarin, enoxaparin, DOAC) within 14 days of bronchoscopy
- Rationale for exposing study participants to the risk of PET-CT imaging: As stated above there are few data in humans that shed light on the CD4 T cell amnestic response in the lung tissue vs draining mediastinal lymph nodes in the context of Mtb exposure. PET-CT imaging is a very sensitive and non-invasive method for visualizing thoracic inflammation. For these reasons, longitudinal PET-CT images will provide critical non-invasive evaluations of cellular activity within the human lung parenchyma and draining mediastinal lymph nodes after exposure to Mtb antigen. In order to obtain evaluation of thoracic inflammation

over time, it will be optimal to perform PET-CT imaging prior to Mtb challenge, at an early time point and a late time point post challenge. The risks of evaluation of thoracic inflammation via repeat PET-CT imaging to the participant will be minimized by the following means:

- Absence of invasive lymph node sampling via endobronchial ultrasound and transbronchial biopsy
- Limitation of the imaging field to include only the thorax and thus reduce overall radiation exposure

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective of this study is to understand the pulmonary immune response to mycobacterial antigens by determining the persistence of antigen-specific immune cells in the airways versus circulation after local bronchoscopic instillation of PPD into the lungs of adults with or without LTBI.	The primary endpoint will be the enumeration of Mtb antigen-specific CD4+ and CD8+ T cell populations in the airway and peripheral blood at early and late time points after bronchoscopic PPD instillation.	This endpoint is chosen to determine the point after directed bronchoscopic exposure to Mtb peptide the antigen-specific T cell response is strongest both locally in the lung and systemically in the blood.
Secondary		
The secondary objective is to characterize the location and persistence of immune cell activity in the pulmonary parenchyma and thoracic lymph nodes with positron emission tomography combined with chest computed tomography (PET-CT) after bronchoscopic PPD instillation in adults with or without LTBI.	The secondary study endpoint of this study is the quantification of FDG SUV throughout all lung fields and mediastinal lymph nodes of LTBI and non-LTBI patients at days -7, +4, and +20 after PPD bronchoscopic instillation.	This endpoint is chosen in order to identify and track over time the geographically anatomic distribution of immune cell activity in the lungs after PPD instillation.
Exploratory		
The exploratory objectives of this study are (a) to perform phenotypic and functional analysis of immune cell populations in the airways vs circulation following bronchoscopic instillation of PPD into the lungs of individuals with or without LTBI, (b) to characterize the production of soluble mediators (i.e. cytokines, lipid mediators, defensins) in BAL	(a) An exploratory endpoint will be the quantification of frequencies of cell surface molecules, intracellular cytokines and transcription factors among immune cell populations in the airways vs circulation following bronchoscopic instillation of PPD into the lungs of individuals with or without LTBI. (b) An exploratory endpoint will involve the	These endpoints are chosen to characterize the cellular phenotype and function of the most influential immune cell populations within the context of pulmonary tuberculosis in the local lung micro-

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
fluid vs blood within the same context.	identification and quantification of soluble mediators in the airway and peripheral blood after PPD instillation in the lungs within the same context.	environment as compared to systemically at empirically selected early and late time points post Mtb antigen exposure.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Hypothesis

The major hypothesis of this single-site pilot study is that recruitment of antigen-specific T cells and innate immune cell subsets following bronchoscopic instillation of purified protein derivative (PPD) from *M. tuberculosis* into the lung will occur primarily in individuals with latent TB infection. These Mtb-specific T cell responses will occur only in the segments into which PPD was instilled and not in the control lung segments. Lastly, pulmonary immune cell activation and number will peak early after PPD instillation and subsequently wane both in the airways and peripheral blood. These responses will be much more pronounced in the local pulmonary micro-environment as opposed to the systemic vasculature.

Using PET-CT imaging after PPD instillation, we hypothesize that it will be possible to identify increased cellular activity in draining thoracic lymph nodes, as well as in the pulmonary parenchyma. Furthermore, we expect that the above signals we are able to discern will progressively wane in the weeks following bronchoscopic PPD instillation.

Longitudinal Study Design

We plan to screen up to 100 non-LTBI controls vs confirmed LTBI to enroll up to 25 individuals in order to have a total of 20 individuals who are concordant on the PPD and IGRA test complete the study as follows:

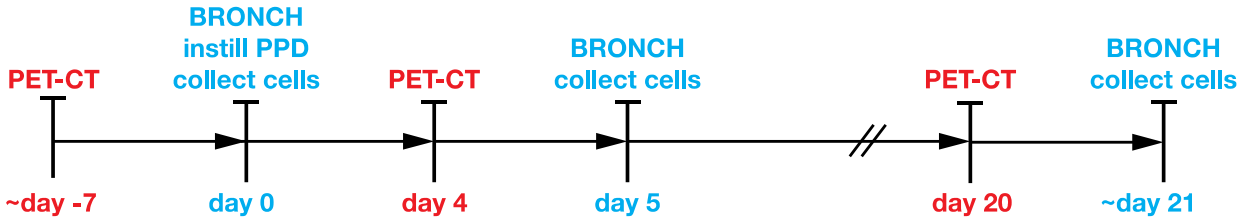
Group A (LTBI Group): 13 PPD + and IGRA + subjects
Group B (non-LTBI Group): 7 PPD - and IGRA - subjects

We seek to enroll individuals at a 2:1 ratio of Group A:Group B in order to maximize the data derived from individuals with latent TB who will mount antigen-specific T cell responses post PPD instillation. This strategy will also decrease variance in Group A via relative oversampling compared to Group B.

Figure 1 outlines the experimental timeline. A baseline PET-CT study of the thoracic cavity will be obtained, and the first bronchoscopy will be performed in the following the week to collect the baseline BAL sample and to instill the PPD as outlined above. Peripheral blood will be collected at the time of each bronchoscopy procedure before the BAL. To examine early immune responses, on approximately day 4 the second PET-CT study will be obtained and the following day a second BAL will be performed, and peripheral blood samples will be collected. BAL will be done first in the control anterior segment of the left upper lobe and then again in the

anterior segment of the right upper lobe. To examine the persistence of this local pulmonary immune response, the next PET-CT image will be obtained on approximately day 20, and the following day 21, repeat blood and BAL samples will be collected as done previously. A repeat IGRA test will be collected on the final visit day of the study to assess for any change in the participant's initial result.

Figure 1. Experimental schematic.



4.2 Scientific Rationale for Study Design

Our study design is similar to that of Silver and colleagues in that we are proposing the evaluation of local airway responses in small groups of non-LTBI controls and confirmed LTBI cases. However, we anticipate that our immunological signals will be stronger due to our confirmation of LTBI status using concordant positive TST and positive IGRA tests, and similarly our confirmation of the status of controls without LTBI using concordant for negative TST and negative IGRA tests. In addition, we will be studying responses to antigen challenges in the upper lobes where most humans develop pulmonary TB, rather than the middle lobe as studied by Silver and colleagues. Furthermore, the novelty of our approach will involve the combination of immunological assays after bronchoscopic instillation of antigens directed to specific lung segments and PET-CT scanning to determine the location and kinetics of responses in the draining lymph nodes. The PET-CT scanning component has the potential to provide the first localization of immunological challenge with non-infectious Mtb antigens in humans.

4.3 Justification for Dose

The PPD bronchoscopic instillation dose of 0.5 tuberculin units (10 uL Tubersol® in 10 mL normal saline) was determined by Silver and colleagues in 2003. They demonstrated that the minimally reactive dose of PPD administered through the airways to elicit the development of alveolar inflammation was a challenge dose of 0.5 TU or 1/10th of the standard TST dose (21).

5 STUDY POPULATION

Eligibility will be determined per two screening visits either in person or via a Telehealth visit. Alternatively, if a test result is available from any other NIH protocol within 60 days of enrollment, that test result can also be used for screening purposes.

5.1 Inclusion Criteria

Adults 18-64 years old will be recruited for the two groups (non-LTBI controls vs confirmed LTBI). The lower limit of this age range is based on the need for invasive bronchoscopic

procedures and exposure to radiation, both of which carry more risk at younger ages. In addition, most adults present with post-primary or reactivation TB that most often occurs radiographically in the upper lobes of the lungs, often with cavitation. Conversely, children and rare adults with primary TB have non-cavitary disease in the lower lobes. The higher limit of this age range is based on the known property of immune senescence, i.e., the waning of the strength of immune responses with advancing age.

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Ability of subject to understand and the willingness to sign a written informed consent document.
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 18 – 64 years of age
4. No significant active medical problems. This would include but not limited to any cardiac disorder (e.g. arrhythmia, valvular disease), pulmonary disease (e.g. asthma requiring chronic medications, chronic bronchitis, emphysema, obstructive sleep apnea), kidney disease (e.g. nephritis, nephrosis), rheumatologic disorder (e.g. inflammatory arthritis), endocrine disorder (e.g. diabetes, thyroid disease), liver disease (e.g. hepatitis), gastrointestinal disorder (e.g. inflammatory bowel disease) or infectious disease (e.g. active tuberculosis).
5. For females of reproductive potential: use of highly effective contraception for at least 1 month prior to screening and agreement to use such a method during study participation
6. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner and agreement to use such a method during study participation

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Pregnancy as assessed by urinary or plasma HCG or breastfeeding
2. History of clinically significant respiratory dysfunction 3 months prior to participating
    a. Evidence of new pulmonary infection
    b. History of any chronic lung infections or chronic lung disease
    c. History of pulmonary hypertension
    d. Need for supplemental oxygen administration at rest
3. Current use or inability to suspend use of anticoagulant therapy including platelet inhibitors (e.g. clopidogrel) within 7 days of study bronchoscopy or systemic anticoagulants (e.g. warfarin, enoxaparin, or DOAC) within 14 days of study bronchoscopy
4. Any symptoms consistent with infection including fever, chills, night sweats, or unexplained weight loss
5. A history of a necrotic reaction to a tuberculin skin test, including during screening
6. A history of human immunodeficiency virus (HIV) infection
7. A history of coughing up blood in the last 3 months
8. Cigarette smoking, vaping or recreational drug use within the past 6 months (self-reported)

9. If there is any discrepancy in tuberculin skin test and Interferon Gamma Release Assay test results (i.e. PPD+ but IGRA- or PPD- but IGRA+)
10. Refusal or inability to undergo bronchoscopy, or a history of poor tolerance of a bronchoscopy
11. If undergoing PET-CT imaging during this study places a participant over his/her annual radiation dose limit. Radiation exposure within the previous 12 months of ≥ 2.3 rem
12. BMI > 40
13. Diabetes
14. Known life-threatening allergic reaction to Tuberculin, Lidocaine, Midazolam, Fentanyl or medications of similar classes
15. Presence of any immunosuppressive diseases, including cancers with the exception of non-melanomatous skin cancer.
16. Use of any systemic immunosuppressive medications, including corticosteroids (e.g., prednisone) or biological agents in the last 6 months prior to enrollment
17. Any medical, psychiatric, social condition, occupational reason or other responsibility, in the judgement of the investigator, that is a contraindication to protocol participation or impairs a participant's ability to give informed consent
18. Positive for COVID-19. (with-in 6 months prior to enrollment self-reported and or via PCR)

5.3 Inclusion of Vulnerable Participants

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines, per Policy 404, for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Employee Research Participation".

5.4 Lifestyle Considerations

Not applicable.

5.5 Screen Failures

Screen failures will be defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an evidence of active clinical illness, or radiation exposure within the last 12 months of $\geq$ 2.3 rem, at the time of screening may be rescreened. Subject's may be eligible to be rescreened to participate at a later date if at the time of enrollment they are found to have an active infection, vaccination, and or have been exposed to radiation within the last 12 months of $\geq$2.3 rem, or met criteria, but subsequently not assigned to the study intervention within 60 days.

If re-screened after 60 days from screening visits, the TST, IGRA, & chest x-ray will not be repeated unless there is a known tuberculosis exposure, including but not limited to travel to an endemic area or direct contact with an individual with known or suspected tuberculosis disease.

The study team may contact the subject at a later date to determine if they would like to continue their participation and complete the screening again.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

The study will be listed on the ClinicalTrials.gov, Clinical Center (CC) Search the Studies, and the CC recruitment website. IRB-approved language will be used in all recruitment messaging and the following options for marketing the study may be used:

- ResearchMatch;
- Official NIH social media accounts (including but not limited to Facebook, Twitter, Instagram);
- Community newspapers, magazines, radio, TV ads and/or other appropriate print or electronic media;
- NIH Listservs (GovDelivery, Clinical Fellows, Post Baccalaureate, OPR Study Volunteer), webpages, or other marketing platforms including Clinical Center TVs (CCTV) messaging;
- Physician partnerships to include health specialists;
- Recruitment post cards and flyers printed will be designed for the study and distributed in the community, around the NIH campus, external partnering healthcare centers/offices, and/or any other locations where potential research volunteers may be found.
- Metro train, bus, station ads (Outfront Media company partnership). All messaging will be IRB approved in advance.

A letter to physicians may be used for electronic (and hard copy as appropriate) distribution to local physicians and specialists. All options mentioned in PSA (i.e. ResearchMatch, publications, etc.) may be used for recruitment. Social media posts may be used by the accounts of the Clinical Center and NHLBI, and/or other official NIH accounts. The Healthy Volunteer (HV) listserv will be used for outgoing communications and a regularly updated HV list will be obtained, all through a close partnership with the CC HV office within the Clinical Center Office of Patient Recruitment and Call Center.

5.6.1 Costs

Not applicable

5.6.2 Compensation

Visits to NIH	**Amount**
Screening Visit #1 – 1hr	**$80**
Screening Visit #2 – 1hr	**$80**
On-Study Visit #1 (Baseline)– 3hrs	$120
On-Study Visit #2 (Day 0)– 5hrs	$120
On-Study Visit #3 (Day 4-5)- 4hrs	$120
On-Study Visit #4 (Day 5-6)– 5hrs	$120
On-Study Visit #5 (Day 20-21)– 4hrs	$120
On-Study Visit #6 (Day 21-22)– 5hrs	$120
Total for Visits	**$880**

Procedures	Amount
Tuberculin skin test (TST) - $50 x 2 2nd test may not be needed	**$100**
History and Physical	$20
EKG - $20 x 4	$80
Blood work – $30 x 4	$120
Research blood work - $30 x 3	$90
Screening chest X-ray	$20
PET-CT - $250 x 3	$750
Radioactive tracer - $80 x 3	$240
Bronchoscopy - $300 x 3	$900
PPD Study drug given with 1st Bronchoscopy	$50
Fentanyl and Midazolam IV admin - $20 x 3	$60
Escort fee for person staying with vol after bronch $20 x3	$60
Total for Procedures	$2,490
Total (Visits and Procedures) - $880 +2,490	$3,370

Recruitment, Selection and Compensation of Research Subjects per Policy 301.

Reimbursement of Travel

Reimbursement for travel will be in accordance with NHLBI travel policy reimbursement for food and lodging will be consistent with NIH and NHLBI guidelines. Car mileage will be reimbursed only if traveling ≥30 miles away at 0.40 cents per mile. Reimbursement will be provided for long distance >50 miles for train and bus only.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

The normal saline control agent (serving as a comparison for PPD administration) for bronchoscopic instillation will be 0.9% sodium chloride solution.
Sterile commercially developed tuberculin PPD will be used for skin-testing and bronchoscopic instillation procedures. Per the Tubersol® Package insert: "Tuberculin Purified Protein Derivative (Mantoux) (PPD) for intradermal tuberculin testing is prepared from a large Master Batch Connaught Tuberculin (CT68) and is a cell-free purified protein fraction obtained from a human strain of *Mycobacterium tuberculosis* grown on a protein-free synthetic medium and inactivated. TUBERSOL is indicated to aid diagnosis of tuberculosis infection in persons at increased risk of developing active disease. It is currently only indicated for intradermal injection.

Endobronchial administration of tuberculin PPD may lead side effects including but not limited to cough, wheezing, bronchospasm, fever, pneumonitis, allergic reaction, or anaphylaxis. The possibility of the occurrence of such side effects will be monitored as detailed below.

This study will be conducted under an Investigational New Drug Application (IND). The IND for Tuberculin Purified Protein Derivative (PPD) TUBERSOL® is 19720.

6.1.2 Dosing and Administration

The PPD bronchoscopic instillation dose of 0.5 tuberculin units (10 uL Tubersol® in 10 mL normal saline) was determined by Silver and colleagues in 2003. They demonstrated that the minimally reactive dose of PPD administered through the airways to elicit the development of alveolar inflammation was a challenge dose of 0.5 TU or 1/10th of the standard tuberculin skin test dose (15).

This instillation will be performed via introduction of a balloon-tipped 5-French pulmonary artery (PA) catheter within the working channel of the bronchoscope, subsequent occlusion of the subsegment of interest, instillation of normal saline control, followed by a saline flush (20). Next, the bronchoscope will be moved to the right lung, where 0.5 tuberculin units (10 microliters of Tubersol®) diluted in 10 cc normal saline will be instilled into the anterior segment of the right upper lobe via a separate 5-French balloon-tipped PA catheter similarly followed by saline flush.

6.1.3 Dose Escalation

Not applicable.

6.1.4 Dose Limiting Toxicity

Not applicable.

6.1.5 Dose Modifications

Not applicable.

6.1.6 Drug Administration

See section 6.1.2

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

Sterile commercially developed TUBERSOL® will be used for skin-testing and bronchoscopic instillation procedures. Please refer to the Tubersol® Package insert.
PPD for clinical and in vitro use will be purchased as Tubersol® from Sanofi Pasteur, 1 Discovery Drive, Swiftwater, PA 18370.

6.2.2 Formulation, Appearance, Packaging, and Labeling

As above in section 6.2.1

6.2.3 Product Storage and Stability

Intact vials will be stored in the refrigerator (2˚C - 8˚C) (36˚F - 46˚F). The vials will be protected from freezing and light exposure in order to maintain product integrity.
Stability studies have demonstrated that PPD containing the stabilizer of Tween 80 and preservative of phenol are stable for at least 3 years at 4 degrees C, 2 years at room temperature (24 degrees C) and even at 37 degrees C are stable for at least 1 year (27). Per Canadian and U.S. regulations PPD will be stored at 2 to 8 degrees C in their original containers. The reconstituted solution should be stored in the refrigerator (2˚C - 8˚C). . The pharmacy will be notified of when the subject will be ready for the procedure and prepare the syringe at this time. The Syringe will be transferred to the procedural unit per the Pharmacy Drug Preparation Manual Standard of Practice (SOP).

6.2.4 Preparation

Tubersol® contains 0.0005% Tween 80 as a stabilizer and 0.28% phenol as a preservative. In order to administer the experimentally established bronchoscopic instillation dose of 0.5

tuberculin units (TU), 10 ul of the commercial Tubersol preparation will be diluted in 10 ml of saline, creating final concentrations of 0.0000025% Tween 80 of and 0.0014% phenol. To ensure sterility dilutions of PPD will be prepared in a laminar flow hood immediately prior to bronchoscopic instillation using previously unopened vials of Tubersol® and sterile saline. For intradermal injection each standard dose of 5 TU will be administered in the standard volume of 0.1 ml using 26G needles.

6.3 Measures to Minimize Bias: Randomization and Blinding

In order to minimize observer and confirmation bias, research specimens collected will be coded so that the principal investigator will be blinded to which group (LTBI vs. non-LTBI) subjects are enrolled, after subject's completion of the schedule of activities but prior to primary data analysis has been completed. Designated AIs and study research nurses will coordinate the process of coding and labeling the samples collected into respective LTBI and non-LTBI groups in a manner that will blind the principal investigator to study group. These specimen code group will only be released to the principal investigator after flow cytometry, and PET-CT data have been collected and undergone primary analysis.

6.4 Study Intervention Compliance

Adherence to the protocol will be assessed by consistent patient presentation to all pre-described appointments including blood collection, PET-CT scans and bronchoscopies.

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the medical record that include concomitant prescription medications, over-the-counter medications and supplements. After the completion of the study protocol, all study participants within the LTBI (PPD +, IGRA +) group will be recommended for referral to their local county health department or an infectious disease outpatient clinic for initiation of LTBI therapy as outlined by the WHO with the regimen selected per the recommendation of their clinician (19).

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- In the event of a single serious adverse event (SAE) or Grade 3 or higher adverse event or laboratory abnormality that is assessed as related or possibly related to the study procedures. Events will be followed until resolution or stabilization.
- If a study participant misses one or more study appointments
- Investigator discretion
- Positive pregnancy test
- Positive COVID-19 PCR

- Current use of anticoagulant therapy including platelet inhibitors (e.g. clopidogrel), or systemic anticoagulants (e.g. warfarin, enoxaparin, or DOAC).

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Subject has completed the study follow-up period
- Death
- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are assigned to a cohort but do not receive the study intervention may be replaced. Subjects who sign the informed consent form and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for one scheduled visit and is unable to be contacted by the study site staff.
The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within three days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

<u>Screening activities performed prior to obtaining informed consent</u>
Minimal risk activities that may be performed before the subject has signed a consent include the following:
• Email, written, in person or telephone communications with prospective subjects
• Review of existing medical records to include H&P, laboratory studies, etc.

Once the research team has identified a potential subject for the study, the subject will be asked to come to the NIH. The study team will consent the subject via in person, telephone and or Telehealth visit platform, allowing time for the subjects to ask questions and make a voluntary decision.
The following tests will be performed to determine eligibility.

- Review of medical history including history of Mtb Exposure
  - History of exposure to persons with active TB
  - History of residence in a TB-endemic country
  - Estimate of time when LTBI most likely occurred
  - Exposure to Bacillus Calmette–Guérin (BCG) vaccine
- Review of concurrent medications and vaccinations performed within the last year
  - Of note, eligibility review and procedures will be completed at least 2 weeks following most recent vaccination

The following tests will be performed after informed consent is obtained to determine eligibility.

- Vital signs
- Complete blood count with diff, acute care panel, hepatic care panel, LDH, International Normalized Ratio (INR), Prothrombin Time (PT), and Partial Thromboplastin Time (PTT) , interferon gamma release assay (IGRA), HIV (I/II), Hepatitis B and C
- Plasma or urinary HCG in female subjects who are of child-bearing age will be collected (after informed consent and within 7 days of any research study procedure)
- Tuberculin skin test (TST) will be performed and read within 48 to 72 hours. A second TST (two-step) may be performed on individuals who have a positive IGRA result, but negative TST result on initial screening. Two-step TST will be performed 1 to 3 weeks after the 1st test.
- Chest X-Ray
- EKG
- COVID-19 PCR

8.2 EFFICACY ASSESSMENTS

8.2.1 Clinical Evaluations

Physical examination
History and physical examination with vital signs (weight, height, BMI, blood pressure, respirations, heart rate, temperature and oxygen saturation) will performed at screening with focus on respiratory and cardiovascular systems. Repeat physical examinations with focus on respiratory and cardiovascular systems with vital sign measurements (blood pressure, respirations, heart rate, temperature and oxygen saturation) will be performed prior to each bronchoscopy.

Radiographic or other imaging assessments.
Chest PET-CT Scan

Subjects will undergo a total of 3 PET/CT scans. A baseline PET/CT of the chest will be performed no more than one week prior to the initial bronchoscopy (10). In order to examine early immune responses, on day 4 post instillation bronchoscopy, a second PET-CT scan will be obtained. Furthermore, in order to examine the persistence of immune cell metabolic activity in the pulmonary parenchyma and thoracic lymph nodes, a PET-CT image will be obtained on approximately day 20 post initial bronchoscopic challenge.

Each participant will be asked to provide an estimate of their radiation exposure within the calendar in order to assess if participation in the study will cause a participant to exceed his/her annual dose limit.

8.2.2 Biospecimen Evaluations

Biological specimen collection and laboratory evaluations.
A peripheral intravenous catheter will be placed to obtain blood for analysis (up to 120 mL). The whole blood volume will be divided into aliquots for myeloid cell flow cytometry analysis, peripheral blood mononuclear cell (PBMC) isolation and laboratory testing. PBMC isolation and subsequent in-vitro stimulation with PPD antigen is detailed below. Blood for clinical serologic testing will be sent to the NIH chemistry lab for analysis via complete metabolic panel (CMP) and c-reactive protein (CRP) and the hematology lab for analysis via complete blood count with differential count (CBC w/ diff) and erythrocyte sediment count (ESR). The amount of blood that may be drawn from adult patients and volunteers (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

In order to assess for any concurrent lower respiratory tract infection in study subjects, a 2 mL aliquot of bronchoalveolar lavage (BAL) fluid from day 0, day 5 and day 21 will be set aside from the research analysis sample. This BAL aliquot will be sent to the NIH Clinical Center Microbiology department for bacterial gram stain and culture in addition to BioFire respiratory pathogen panel for detection of the following viral and bacterial organisms: Adenovirus, Coronavirus HKU1/NL63/229E/OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza B, Parainfluenza, Respiratory Syncytial Virus, Bordetella pertussis, Chlamydia pneumoniae, and Mycoplasma pneumoniae.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

Special assays or procedures required
Blood will be collected into heparinized Green Top tubes on the day of each bronchoscopy (days 0, 5 and 21). Peripheral Blood Mononuclear Cell (PBMC) and BAL cells will be isolated, stimulated and cell surface molecules, intracellular cytokines and transcription factors will be evaluated via flow cytometry. Peripheral blood and BAL soluble mediators will be analyzed via multiplex kits, mass spectrometry or other techniques.

8.2.4 Samples for Genetic/Genomic Analysis

Not applicable.

8.3 SAFETY AND OTHER ASSESSMENTS

First participants will undergo history and physical assessment, blood or urine pregnancy test and a confirmatory TB skin test, and have blood drawn for an interferon gamma release assay (IGRA; Quantiferon Gold In-Tube, Qiagen) screening. Subjects will undergo a chest x-ray (CXR) to rule out for active disease. This is to ensure their eligibility prior to undergoing PET/CT scans, and bronchoscopies.
Eligible study subjects will be given a username and password to access a web-based patient self-reporting diary to record any evidence of self- assessed adverse events. At each study visit this will be reviewed with the subject by a designated research study personnel. If a subject reports a possible serious adverse event, then the subject will be contacted immediately for review of event(s) by designated research study personnel. Subjects will also be directed to contact research study nurse if they have any concerns or questions.

Biological specimen collection and laboratory evaluations.
Bronchoscopy, BAL and Bronchoscopic Instillation
Study subjects will undergo three bronchoscopies and bronchoalveolar lavages for the evaluation of airway and alveolar immune cells.
The study subject will be required to be without solid food or drink after midnight or six hours prior to each bronchoscopy.
First, topical anesthesia will be achieved via administration of inhaled medications containing lidocaine. Following completion of local anesthesia, sedative medications will be administered to achieve a level of moderate sedation.
A bronchoscope will be introduced via the trans-oral or trans-nasal route. Lidocaine will be instilled through the bronchoscope at the levels of the epiglottis, vocal cords, carina and mainstem bronchi to provide additional anesthesia during the procedure. A baseline (prior to control or PPD instillation) bronchoalveolar lavage (BAL) will be performed in the left superior lingular segment of the left upper lobe. The control instillation of normal saline will then be placed into the anterior segment of the left upper lobe. This instillation will be performed via introduction of a balloon-tipped 5-French pulmonary artery (PA) catheter (26). Next, the bronchoscope will be moved to the right lung, similarly, a baseline BAL will be performed in the right middle lobe medial segment. Immediately afterwards, tuberculin purified protein derivative will be instilled into the anterior segment of the right upper lobe via a separate 5-French balloon-tipped PA catheter. The bronchoscope will then be removed and the subject will recover and be monitored. If a subject requires additional monitoring they will be transferred to the Day Unit of the Clinical Center. Signs and symptoms for expected and unexpected responses from bronchoscopy and PPD instillation will be assessed during this time. At time of discharge their vital signs must return to baseline, the subject must be able to eat and drink without difficulty and have a responsible adult available for transport from the Clinical Center. Each participant will be called the following day to assess their respiratory status and evaluate for the development of any new pulmonary symptoms. If necessary, they will be re-evaluated at the Clinical Center by Dr. Fennelly.
Provide Study Monitoring Plan:

We will use the Common Terminology Criteria for Adverse Events for Adverse Events (CTCAE) Version 5.0 to assess for any adverse events related to endobronchial PPD instillation. Adverse events (AE) will be graded by their level of severity (Grade 1 mild, Grade 2 moderate, Grade 3 severe, Grade 4 life-threatening consequences, Grade 5 Death related to AE). We will specifically assess for evidence of respiratory-related adverse events commonly associated with bronchoscopy and/or endobronchial administration of a non-endogenous agent which includes but is not limited do: dyspnea, cough, sore throat, stridor, wheezing, bronchospasm, hypoxia, laryngeal inflammation, laryngospasm, pneumonitis, or pneumothorax. We will similarly assess for evidence of systemic immune- or infectious-related adverse events including but not limited to: allergic reaction, anaphylaxis, bronchial infection, lung infection, pharyngitis and/or pleural infection.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

This study will utilize the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) for toxicity and adverse event reporting. A copy of the CTCAE v5.0 can be downloaded from the https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
AEs will be recorded, verified, and followed until satisfactory resolution or stabilization. Severity definitions found in the CTCAE v5.0 will be used for grading the severity (intensity) of AEs:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".
- **Life-threatening** – Events with life-threatening consequences in which urgent intervention is indicated
- **Death** – Death related to an adverse event

8.4.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.4.3.3 Expectedness

The principal investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. The expected risks will be primarily related to bronchoscopy and endobronchial manipulations. These risks include: dyspnea, cough, sore throat, stridor, wheezing, bronchospasm, hypoxia, laryngeal inflammation, laryngospasm, pneumonitis, pneumothorax, allergic reaction, and anaphylaxis. We expect some vasovagal symptoms during blood draws (expected frequency 50%) and transient bruising at the site of blood draws (expected frequency 50%).

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.
All AEs including clinically significant laboratory tests, local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product/research procedure (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The principal investigator 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 research procedure or until resolution or stabilization of an SAE whichever comes later . At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization via in person, telephone and or e-mail.

8.4.5 Adverse Event Reporting

The study investigator will be responsible for reporting all AEs, regardless of attribution, to the sponsor at the time of NIH IRB continuing review and FDA annual report.

If at any time the study investigator believes that a frequency of an AE is greater than anticipated, the investigator will report these findings to the sponsor within 7 days.

8.4.6 Serious Adverse Event Reporting

The study investigator will report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.
All serious adverse events (SAEs) will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the participant is stable.
The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.
All SAEs will be reported to the DSMB at the time of review. However, if an SAE is considered, unexpected and related to the intervention, the study investigator will notify the DSMB of the event within 48 hours.

8.4.7 Events of Special Interest

Any incidental radiographic findings discovered on PET-CT imaging will be reported to a study subject no later than 7 days after the PI first learns of the result.

8.4.8 Reporting of Pregnancy

If the serum or urinary beta-HCG result of a female participant is positive at the time of screening, the individual will be informed of this result and excluded from the study.
All female subjects will receive pregnancy testing prior to radiation exposure. Should a subject find out that she became pregnant while participating in this study, she should notify the team immediately. The team will provide counseling on the risks of radiation to the fetus. The investigators will report the pregnancy to the IRB and NHLBI Clinical Director at the Continuning Review as a reason subject was withdrawn early from the study

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

- *Primary Endpoint:*
  We hypothesize that antigen-specific CD4+ T cells response will be highest in the BAL five days post challenge in LTBI subjects alone and may subsequently wane 21 days post challenge.
- *Secondary Endpoint:*
  We hypothesize that there will be significant FDG-avidity within the lung parenchyma and adjacent thoracic lymph node within the area of the lung (right upper lobe) in which PPD is selectively instilled. We expect that the SUV quantities will be most pronounced in LTBI subjects at day 4 post challenge and subsequently wane at day 20.
- *Exploratory Endpoints:*

We hypothesize that the phenotype and function of Mtb-specific T cells in the BAL will differ from that of circulating T cells. We hypothesize that bronchoscopic instillation of PPD will induce production of TH1 predominant pro-inflammatory cytokines, eicosanoid lipid mediators and defensins in the local airway environment more so than the systemic vasculature. We hypothesize that these soluble mediators will be primarily present in LTBI subjects on day 5 post challenge and wane at day 20.

9.2 SAMPLE SIZE DETERMINATION

This is a pilot study which will be hypothesis-generating in nature. For this reason we will not be pursuing the formal sample size calculation that corresponds to a specified statistical power. However, the sample size of 13 confirmed LTBI cases and 7 confirmed non-LTBI controls (a total of 20 subjects) is slightly larger than those of the original studies of the current bronchoscopic instillation model pioneered by Silver and colleagues (21-23). Silver's 2003, 2005 and 2011 PPD bronchoscopic instillation studies included a total of 9, 15, and 14 total subjects respectively. Each study was able to demonstrate a statistically significant increase in the frequency of Mtb-specific CD4+ T cells in the BAL of LTBI subjects as compared to the BAL of non-LTBI controls within each study. Furthermore, we anticipate that our immunological signals will likely stand out due to confirmation of LTBI status using concordant TST and IGRA testing. We will enroll up to an additional 5 subjects in order to achieve 20 completed studies.

9.3 POPULATIONS FOR ANALYSES

Not applicable.

9.3.1 Evaluable for toxicity

Not applicable.

9.3.2 Evaluable for objective response

Not applicable.

9.3.3 Evaluable Non-Target Disease Response

Not applicable

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

Statistical analysis will be performed in R version 3.6.1 or later (CRAN.R-Project.org). For the analysis of primary and secondary endpoints, mixed effects models with repeated measure will be used to compare the LTBI and non-LTBI responses after bronchoscopic PPD instillation. For the primary hypothesis, the cell count for Mtb antigen-specific CD4+ and CD8+ T cells will be used as the response variable. Two separate statistical models will be entertained to explain the local pulmonary response and systemic vasculature, respectively. To guard against non-normal responses, a suitable data transformation on the response variable, e.g., log transform, will be performed if the original scale is highly skewed.

In regard to PET CT analysis, calculations for standardized uptake value will be made and normalized for scan differences using OsiriX MD (11). For the secondary hypothesis, the FDG SUV values from the PET CT image, measured at days -7, +4, and +20 days will be used as response in similar mixed effects models to study the group difference in average immunologic responses.
For the exploratory hypotheses, both numerical and graphical summary tools will be used to study the phenotypic and functional analysis of immune cell populations in the airways and circulation of individuals with or without LTBI. Also, descriptive statistics will be used to characterize the production of soluble mediators in BAL fluid and blood.

9.4.2 Analysis of the Primary and Secondary Endpoints

For the analysis of primary and secondary endpoints, mixed effects models with repeated measure will be used to compare the LTBI and non-LTBI responses after bronchoscopic PPD instillation.
For the primary hypothesis, the cell count for Mtb antigen-specific CD4+ and CD8+ T cells will be used as the response variable. Two separate statistical models will be entertained to explain the local pulmonary response and systemic vasculature, respectively. To guard against non-normal responses, a suitable data transformation on the response variable, e.g., log transform, will be performed if the original scale is highly skewed.

9.4.3 Safety Analyses

N/A

9.4.4 Baseline Descriptive Statistics

In this pilot study, various descriptive statistics will be used to describe and compare baseline patient demographics per group.

9.4.5 Planned Interim Analyses

None planned at this time

9.4.6 Sub-Group Analyses

The primary, secondary and exploratory endpoints will be analyzed within group stratified for age in order to determine if there is evidence of a differential response in the setting of immunosenescence.

9.4.7 Tabulation of individual Participant Data

Each individual participant data will be listed by measurement evaluated, location of measurement acquisition and day of measurement acquisition relative to bronchoscopic challenge date.

9.4.8 Exploratory Analyses

For the exploratory hypotheses, both numerical and graphical summary tools will be used to study the phenotypic and functional analysis of immune cell populations in the airways and

circulation of individuals with or without LTBI. Also, descriptive statistics will be used to characterize the production of soluble mediators in BAL fluid and blood.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Informed Consent Processes and Procedures
Informed consent will be conducted following OHSRP Policy 301. An IRB-approved consent form, will be provided to the subject electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone, telehealth or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the subject in a private setting. The subject will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.
A signed and dated informed consent document will be obtained by any investigator authorized to consent (See Key Study Personnel) prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.
When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.
During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.
Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic). When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.
Whether in person or remote, the privacy of the subject will be maintained.
The fully signed informed consent document will be stored in the electronic medical record, and the subject will receive a copy of the signed informed consent document.

10.1.2 Consent for minors when they reach the age of majority

Not applicable.

10.1.3 Telephone and or Telehealth platform consent

Participants may be contacted by telephone and or Telehealth NIH- approved platform per Policy 303 to obtain informed consent. For initial consent and for subjects actively participating in the study who may need to be re-consented due to changes to the informed consent or procedures.

10.1.4 Telephone child assent

Not applicable

10.1.5 Participation of Subjects who are/become Decisionally Impaired

Not applicable. Adults who are unable to provide initial informed consent will be excluded.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
  a. A single SAE in a subject that is assessed as related or possibly related to the study drug
  b. Grade 3 or higher laboratory abnormality in 3 or more subjects with a reasonable possibility of a causal relationship to the administration of study drug
  c. Grade 3 or higher with the same or similar Adverse Event in 2 or more subjects assessed as possibly related to study drug.
  d. Will restart study if investigation determines study drug not to be the cause
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a

secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.
Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Barber Lab data server. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the at the Barber Lab data server.
To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

After analyzing the biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research will be stored in the Principal Investigator's laboratory freezers in a coded manner to ensure data protection. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB. Any future research use of biospecimens not defined in the research protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use where results will not be returned to the Principal Investigator does not require IRB review. Refusal of a research subject participant to allow for future use of biospecimens will be honored.

10.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least annually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter.

10.6 CLINICAL MONITORING

Principal Investigator:
Accrual will be monitored by the principal investigator who will provide oversight and will monitor accrual and safety data. The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records) readily available for inspection by

the local IRB and the NHLBI Office of the Clinical Director staff for confirmation of the study data.

The monitoring of this study will be conducted by clinical research associates (CRAs)/monitors employed by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP) and applicable guidelines (ICH-GCP) are being followed. Monitoring will be conducted according to the OCD schedule. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the site monitors, and the NHLBI staff for confirmation of the study data.

10.7 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Data Management
The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The PI, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes and investigations as well as from outside medical records. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from outside physicians will be entered into the system. Research data will be prospectively collected by authorized personnel and entered into an NHLBI, 21 CFR 11 compliant, database which will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.
We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, or as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to a NHLBI Program Official. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.
Reports to the CD:
The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:
National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 10 years after the completion of the primary endpoint by contacting Kevin Fennelly.

10.10.2Genomic Data Sharing Plan

N/A

10.11 COLLABORATIVE AGREEMENTS

Not applicable.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NHLBI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
BAL	Bronchoalveolar Lavage
BALF	Bronchoalveolar Lavage Fluid
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CMP	Complete Metabolic Panel
CRF	Case Report Form
CRP	C-Reactive Protein
CXR	Chest X-Ray
DSMB	Data Safety Monitoring Board
EKG	Electrocardiogram
eCRF	Electronic Case Report Forms
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IGRA	Interferon Gamma Release Assay
IND	Investigational New Drug Application
IRB	Institutional Review Board
LTBI	Latent Tuberculosis Infection
LUL	Left Upper Lobe
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
Mtb	Mycobacterium tuberculosis
NCT	National Clinical Trial
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PET-CT	Positron Emission Tomography-Computed Tomography
PI	Principal Investigator
PPD	Purified Protein Derivative
PT	Prothrombin Time
PTT	Partial Thromboplastin Time

QA	Quality Assurance
QC	Quality Control
RML	Right Middle Lobe
RUL	Right Upper Lobe
SAE	Serious Adverse Event
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SUV	Standardized Uptake Value
TST	Tuberculin Skin Test
UP	Unanticipated Problem
US	United States

12 REFERENCES

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