

A Study of Immunologic and Microbiologic Parameters of *M. tuberculosis* Exposure and Infection in the Lungs of Adult Household Contacts of Patients with Pulmonary Tuberculosis

Sponsor Protocol Number: 16-0063

Sponsored by:

National Institute of Allergy and Infectious Diseases (NIAID),
National Institutes of Health (NIH)

Principal Investigators:

W. Henry Boom, MD
Harriet Mayanja-Kizza, MBChB, MMed, PhD

Draft or Version Number: Version 3.0

Day Month Year
10 APR 2019

Statement of Compliance

This study will be conducted as outlined in the protocol in accordance with the following guidelines and regulations. These guidelines and regulations include, but are not limited to:

- Compliance with the International Council on Harmonization (ICH) E6: Good Clinical Practice (GCP): Consolidated Guideline and the applicable regulatory requirements;
- Compliance with the United States Code of Federal Regulations (CFR) applicable to clinical studies (Title 45 CFR Part 46 and Title 21 CFR including Parts 50 and 56 concerning informed consent and Institutional Review Board (IRB) regulations, 21 CFR 312);
- Completion of Human Participants Protection Training;
- NIAID Clinical Terms of Award.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator at CWRU:

Signed:

Date:

W. Henry Boom, MD

Principal Investigator in Uganda:

Signed:

Date:

Harriet Mayanja-Kizza, MBChB, MMed, PhD

Table of Contents

Statement of Compliance.....	2
SIGNATURE PAGE	3
Table of Contents.....	4
Listing of Abbreviations	7
Protocol Synopsis	8
Outline of Study Visits.....	10
1.0 KEY ROLES.....	11
2.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE	13
2.1 Background Information	13
2.1.1 Background	13
2.1.2 Host Immunity to TB.....	13
2.1.3 Anti-MTB Immunity in the Lung	14
2.1.4 Bronchoscopy and Bronchoalveolar Lavage (BAL) as Research Tool	14
2.2 Rationale	14
2.3 Study Objectives	15
2.4 Potential Risks and Benefits	16
2.4.1 Potential Risks	16
2.4.1.1 Bronchoscopy and Bronchoalveolar Lavage	16
2.4.1.2 Blood Draws	18
2.4.1.3 Chest X-rays.....	18
2.4.1.4 Confidentiality.....	19
2.4.1.5 Lidocaine Administration.....	19
2.4.2 Potential Benefits	19
3.0 OBJECTIVES.....	19
3.1 Hypothesis and Objectives.....	19
4.0 STUDY DESIGN	20
5.0 STUDY POPULATION.....	20
5.1 Selection of the Study Population	21
5.2 Inclusion and Exclusion Criteria	21
5.2.1 Enrollment Inclusion Criteria	21
5.2.2 Enrollment Exclusion Criteria	21
6.0 STUDY PROCEDURES AND EVALUATIONS.....	22
6.1 Study Procedures.....	22
6.1.1 Clinical Characteristics, Medical History, Targeted Physical Exam, Vital Signs and SpO2	22
6.1.2 Blood Collection	22
6.1.3 Urine Collection.....	22
6.1.4 HIV Testing	23
6.1.5 Chest Radiographs	23
6.1.6 Bronchoscopy and BAL Procedure	23
6.1.7 Tuberculin Skin Testing (TST)	24

6.2 Laboratory Evaluations	24
6.2.1 Specimen Collection, Handling, and Storage	24
6.2.2 Laboratory Procedures.....	24
6.2.2.1 Routine Laboratory Measurements	24
6.2.2.2 Interferon-gamma Release Assay (IGRA)	25
6.2.2.3 Immune Function Assays.....	25
6.2.2.4 BAL Fluid Analysis	26
7.0 STUDY SCHEDULE	27
7.1 Initial Screening Visit.....	27
7.2 Baseline Eligibility Assessment.....	27
• Counseling and confirmation of consent for bronchoscopy and bronchoalveolar lavage	27
• Medical history, targeted physical examination, vital signs and SpO2.....	27
• Blood draw for the following:	27
○ Complete blood count with platelet count	27
○ PT and aPTT	27
• Urine pregnancy test (for women of child-bearing potential).....	27
7.4 Bronchoscopy Visit.....	28
7.5 Post-Bronchoscopy Visit	28
7.6 Criteria for Discontinuation or Withdrawal of a Participant from the Study	29
8.0 ASSESSMENT OF OUTCOME MEASURES	29
9.0 SAFETY ASSESSMENT AND REPORTING	30
9.1 Definition of Adverse Event (AE).....	30
9.2 Definition of Serious Adverse Event (SAE)	32
9.3 Reporting Procedures	32
9.3.1 Adverse Event Reporting	32
9.3.2 Serious Adverse Event Reporting	33
9.3.3 Detection of Adverse Events and Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings	33
9.3.4 Type and Duration of the Follow-up of Participants After Adverse Events.....	33
9.4 Halting Rules.....	33
9.5 Safety Oversight via Independent Safety Monitor.....	34
10.0 CLINICAL MONITORING	35
10.1 Site Monitoring	35
11.0 STATISTICAL CONSIDERATIONS	35
11.1 Study Outcome Measures.....	35
11.2 Sample Size Considerations	35
Twenty-five participants in each cohort of LTBI, CNVT and RLTBI, will be enrolled into the study. Comparison of IFN-gamma levels between infected (LTBI and/or CNVT) and RLTBI will be an unmatched comparison of means (t-test). The power is based on a 2-sided test with $\alpha=0.05$. The null hypothesis is no difference between group means. IFN-gamma means and standard deviations in the power calculation were based on a previous study by Mahan et al. [28].	35
11.3 Participant Enrollment and Follow-Up.....	36
11.4 Analysis Plan.....	36
12.0 ACCESS TO SOURCE DATA/DOCUMENTS	36

13.0 QUALITY CONTROL AND QUALITY ASSURANCE.....	37
13.1 Quality Control and Assurance Management	37
14.0 ETHICS/PROTECTION OF HUMAN PARTICIPANTS	37
14.1 Ethical Standard.....	37
14.2 Institutional Review Board (IRB)	37
14.3 Informed Consent.....	37
14.4 Exclusion of Children	38
14.5 Participant Confidentiality.....	38
14.6 Cost/Participant Compensation/Research Related Injuries	38
14.7 Future Use of Stored Specimens	39
15.0 DATA HANDLING AND RECORD KEEPING.....	39
15.1 Data Management Responsibilities.....	39
15.2 Data Capture Methods	39
15.3 Types of Data.....	39
15.4 Timing/Reports.....	40
15.5 Study Records Retention	40
15.6 Protocol Deviations	40
16.0 PUBLICATION POLICY	40
17.0 References.....	42
APPENDIX A. TIME AND EVENTS SCHEDULE	45
APPENDIX B. CONSENTS	46

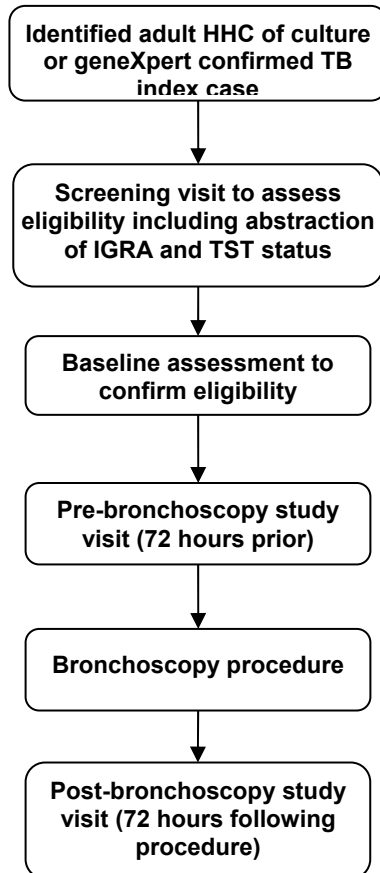
Listing of Abbreviations

AE	Adverse event
ALT	Alanineaminotransferase
aPTT	Activated partial thromboplastin time
BAL	Bronchoalveolar lavage
CBC	Complete blood count
CNVT	Converter from TST and IGRA negative to IGRA positive
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CWRU	Case Western Reserve University
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
ELISA	Enzyme-Linked immunosorbent assay
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus-1
ICH	International Conference on Harmonization
IDI	Infectious Disease Institute
IGRA	Interferon-gamma release assay
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LTBI	Latent M. tuberculosis infection
MTB	Mycobacterium tuberculosis
MU	Makerere University
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NPO	Nil per os (nothing by mouth)
NTLP	National Tuberculosis and Leprosy Programme
QMP	Quality Management Plan
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PI	Principal Investigator
PT	Prothrombin time
RLTBI	Resistance to LTBI
SpO2	Blood oxygen saturation by pulse oximetry
SAE	Serious adverse event
TB	Tuberculosis
TBRU	Tuberculosis Research Unit at CWRU
TST	Tuberculin skin test
UCRC	Uganda-CWRU Research Collaboration
UHCMC	University Hospitals of Cleveland Medical Center
WBC	White blood cell

Protocol Synopsis

Title	A Study of Immunologic and Microbiologic Parameters of <i>M. tuberculosis</i> Exposure and Infection in the Lungs of Adult Household Contacts of Patients with Pulmonary Tuberculosis
Population	18 to 50 year old Ugandan adults who reside in Kampala, Uganda with an individual with active TB disease, and do not show signs and symptoms of active TB disease themselves
Participating Sites	<u>Study site</u> Uganda-Case Western Reserve University (CWRU) Research Collaboration TB Project Clinic at Mulago Hospital and Mulago Hospital, Kampala, Uganda <u>Coordinating Center</u> Tuberculosis Research Unit at CWRU 10900 Euclid Avenue Cleveland, Ohio 44106-4984, U.S.A.
Hypothesis	Innate and adaptive immune responses in the lung control MTB after exposure and infection with aerosolized bacilli. Therefore, comparing innate and adaptive immune responses in the lungs of adult household contacts of patients with pulmonary TB who have developed latent or recent MTB infection (LTBI or CNVT), to those of persons who resist development of LTBI (RLTBI) will provide insight into protective immunity against MTB in the lung. Understanding the unique human protective immune responses to MTB in the lung is critical for development of new TB vaccines and identification of biomarkers of protective immunity against MTB in the lung.
Objectives & Outcomes	<ol style="list-style-type: none"> 1. To define innate and adaptive immune responses to MTB in the lung following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive tuberculin skin test (TST) and positive Interferon-gamma release assay (IGRA) (i.e. persons with LTBI or who recently converted from TST and IGRA negative to IGRA positive (CNVT)) to those who are TST and IGRA negative (i.e. persons with RLTBI). 2. To define innate and adaptive immune responses in the peripheral blood following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1. 3. To compare the rate of positive MTB culture in BAL specimens of healthy adult household contacts of persons with active pulmonary TB of those with a positive TST and/or positive IGRA (i.e. LTBI or CNVT) to those who are TST and IGRA negative (i.e. RLTBI) in parallel with Objective 1. 4. To determine if MTB molecules (e.g. proteins, glycolipids, lipids, DNA) can be detected in BAL of healthy adult household contacts of persons with active pulmonary TB who have a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) and in those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with

	Objective 1.
Study Design	Cross-sectional observational adult TB household contact study to assess host immunological responses in the lungs and peripheral blood of HIV seronegative adults following exposure to an adult household member with culture-confirmed or GeneXpert positive pulmonary TB.
Study Duration	<p><u>Study duration:</u></p> <p>The projected time to enroll all participants and complete this study is 3 years.</p> <p><u>Individual participant duration of follow-up:</u></p> <p>Adult TST and IGRA negative (RLTBI) and positive household contacts without active TB (LTBI and CNVT) will be seen within 72 hours before bronchoscopy and followed for 3 days after bronchoscopy.</p>
Population & Sample Size for both Part I and Part II	<p>Total HIV seronegative, asymptomatic adult household contact participants undergoing bronchoscopy – 75</p> <ul style="list-style-type: none"> • 25 TST and IGRA positive contacts (LTBI) • 25 TST and IGRA negative contacts (RLTBI) • 25 TST and IGRA negative contacts who converted to IGRA positive and remained asymptomatic (CNVT) within 6 months prior to screening for this bronchoscopy study
Study Procedures	Adult household contacts with either a positive or negative TST and IGRA at baseline will have blood drawn and undergo bronchoscopy with BAL. Participants undergoing bronchoscopy will have a pre and post bronchoscopy study visit within 72 hours before and after bronchoscopy.
Host Immunologic, Microbiologic and Genomic Parameters to Be Studied	<p><i>Immunologic and Other Host Response Parameters:</i></p> <ul style="list-style-type: none"> • Cytokine and mRNA levels from BAL fluid and peripheral blood samples • Phenotypic analysis of cells in BAL fluid and peripheral blood samples • T cell responses to <i>M. tuberculosis</i> antigens in BAL fluid and peripheral blood samples <p><i>Microbiological Parameters:</i></p> <ul style="list-style-type: none"> • Presence of viable <i>M. tuberculosis</i> by culture of BAL fluid • Presence of <i>M. tuberculosis</i> nucleotides, proteins and (glyco-lipids) in BAL fluid
Statistical & Analytic Plan	The study is powered to detect differences in cytokine levels between infected (LTBI and CNVT) and RLTBI groups, and between BAL fluid and peripheral blood based on estimates from a previous study. Other measures will be analyzed using exploratory and integrative methods to detect trends and patterns for further validation.

Outline of Study Visits

1.0 KEY ROLES

For questions regarding this protocol, please contact Ms. Robin Mason at the NIH sponsoring office.

Sponsoring Representative(s)**Program Officer**

Robin M. Mason, MS, MBA
Respiratory Disease Branch/DMID/NIAID/NIH
5601 Fishers Lane, 8E34
Bethesda, MD 20892-9825
Phone: +1-240-627-3310
Fax: +1-301-496-8030
RMason@niaid.nih.gov

Medical Officer

Mamodikoe Makhene, MD
Respiratory Disease Branch/DMID/NIAID/NIH
5601 Fishers Lane, Room 8E24
Rockville, MD 20892-9825
Phone: +1-240-627-3309
Fax: +1-301-496-8030
mmakhene@niaid.nih.gov

Institutions / Contact Person(s)

W. Henry Boom, MD, Principal Investigator
TB Research Unit at CWRU
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106-4984
Phone: +1-216-368-1949
Fax: +1-216-368-0105
whb@case.edu

Harriet Mayanja-Kizza, MBChB, MMed, PhD, Uganda/Site Principal Investigator
Uganda-CWRU Research Collaboration
Makerere University, College of Health Sciences
Kampala, Uganda
Phone: +256-414-534-262
Fax: +256-414-533-531
hmk@mucwru.or.ug

John L. Johnson, MD, Co-Investigator
TB Research Unit at CWRU
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106-4984
Phone: +1-216-844-2646
Fax: +1-216-844-0601
jlj@case.edu

Moses Joloba, MBChB, MMed, PhD, Co-Investigator
Uganda-CWRU Research Collaboration
Makerere University, College of Health Sciences
Kampala, Uganda
Phone: +256-414-541-830
Fax: +256-414-533-531
mlj10@case.edu

William Ofuti Worodria, MBChB, MMed, PhD, Co-Investigator
Makerere University
Department of Medicine / College of Health Sciences
PO Box 7072
Kampala, Uganda
Phone: +256-414-534-262
Fax: +256-414-533-531
worodria@yahoo.com

Rejani Lalitha, MBChB, MMed, Co-Investigator
Makerere University
Department of Medicine / College of Health Sciences
PO Box 7072
Kampala, Uganda
Phone: +256- 793-518-080
Fax: +256-414-533-531
rejaniopio@yahoo.com

2.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Background

The World Health Organization reported in 2018 that an estimated 1.7 billion people world-wide were infected with *M. tuberculosis* (MTB) [1]. Of TB cases, 9% were persons co-infected with HIV, and 1.6 million deaths occurred worldwide in 2017. Uganda reported 47,170 new TB cases in 2017, but no longer remains among the 22 high TB burden countries globally [1], with the African region contributing 25% overall to the world's cases during 2017. The high prevalence and mortality of TB is due, in part, to a lack of reliable rapid diagnostic tests and easy to deliver, effective treatment. Standard short course chemotherapy for drug-susceptible TB requires administration of multiple drugs for 6 months. Treatment of patients with drug-resistant TB is even more complex and costly, requiring treatment for 18 or more months and is associated with many side effects. A better understanding of the pathogenesis of TB, leading to an effective vaccine and better treatment, is urgently needed.

The natural history of TB reflects a multi-stage process. In the first stage, TB naive individuals come in contact with an active case of pulmonary TB who is coughing out infected droplet nuclei containing viable tubercle bacilli. Uninfected individuals inhale these droplet nuclei into the lung where pulmonary immune defenses are initially confronted with MTB. Some individuals develop active TB while most contain the infection and become latently infected with MTB (LTBI). A small number appear able to clear or control the infection without developing a T cell response to MTB as measured by TST and IGRA, i.e. they resist development of LTBI (RLTBI) despite extensive and intensive exposure to MTB. These individuals have recently been identified in the Kawempe TB household contact study and are of particular interest because they likely have unique immune defenses against MTB [2]. Most persons with LTBI have controlled the infection but not eliminated it and thus remain at risk of developing active TB for the rest of their lives.

The risk of developing active TB is highest soon after infection and then declines. The incidence of active TB, in the absence of preventive therapy, in the first year after infection is 1,220 per 100,000 persons [61, 3]. The risk declines within 2-5 years to a rate of 300 per 100,000 persons.

While risk factors for transmitting TB have been well documented, very little is known about why some people develop active TB, while others are able to resist and/or contain the infection. The underlying immunologic and genetic basis for host susceptibility to infection and disease is poorly understood. Although the lung is the primary portal for nearly all MTB infection and is the most frequent site of active disease, the mechanisms that allow progression of infection to active TB and those ultimately leading to protective immunity are incompletely understood. One of the main reasons for this lack of knowledge is that pulmonary immune defenses are difficult to study without directly sampling the lung. Recent failure of a novel TB vaccine, which elicited immune responses measurable in peripheral blood, illustrates the critical gap in knowledge of protective immune responses in the human lung that successfully control and (possibly in some) eliminate MTB after infection [4].

2.1.2 Host Immunity to TB

The first line of host pulmonary immune defense against MTB is mediated by alveolar macrophages that engulf tubercle bacilli, process them and present antigens on their cell surface in conjunction with the major histocompatibility cell (MHC) molecules. This interaction activates CD4+ and CD8+ T-cells and results in the release of cytokines interleukin-2 (IL-2) and interferon gamma (IFN- γ) that are central for control of MTB. IL-2 is released by CD4+ T cells, resulting in expansion of antigen-specific CD4+ and CD8+ T-cells, i.e. amplification of the immune response. Activated T-cells release IFN- γ , activating macrophages that control intracellular growth of MTB. Other pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) interleukin-6 (IL-6) and interleukin-12 (IL-12) contribute to the activation of T cells and granuloma formation [5].

In contrast, inhibitory cytokines and regulatory T cells (Treg) may limit the production of pro-inflammatory cytokines by effector T cells. This response is mediated through interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β) [6, 7]. These data, derived primarily from peripheral blood mononuclear cells, indicate that effector Th1 responses are necessary but not sufficient to contain MTB replication. How these human Th1 immune responses are generated and function in the lung is not understood and represents a critical gap in our knowledge of protective immunity to MTB which is essential for TB vaccine development and identification of key correlate and surrogate biomarkers of protection against MTB.

How pulmonary and systemic immune responses correlate or whether lung based microbiological parameters may be more sensitive markers for predicting clinical outcomes following exposure and infection with MTB has not been determined. It is also unknown how immune responses differ among people who develop active disease, versus those who are able to contain MTB, or resist infection all together.

2.1.3 Anti-MTB Immunity in the Lung

Bronchoalveolar lavage (BAL) is a method to directly sample immunological, biochemical and cellular processes in the airways and lungs. Studies of pulmonary immune responses in MTB infected persons are limited. In persons with latent MTB infection or recent MTB exposure, MTB-specific CD4+ and CD8+ T cells are increased in the lung compared to blood without substantial changes in overall BAL cell distribution of T cells and macrophages than in MTB uninfected persons. BAL from household contacts of TB patients in Mexico had evidence of *in vivo* activation with T cell activation and recruitment of immature mononuclear phagocytes, possibly recently arrived blood monocytes. BAL and peripheral blood responses differed in MTB infection and disease. Thus it is critical to identify those responses in peripheral blood that reflect protective immune responses in the lung [8].

2.1.4 Bronchoscopy and Bronchoalveolar Lavage (BAL) as Research Tool

Fiberoptic Bronchoscopy in Chest Medicine

Fiberoptic bronchoscopy was introduced into clinical chest medicine in the 1960s and is used to diagnose, treat and study pulmonary diseases at hospitals and clinics worldwide [9-16]. Fiberoptic bronchoscopy has been safely and extensively used in a wide variety of lung diseases including asthma [17-20], COPD [19], interstitial lung diseases (rheumatoid arthritis, scleroderma, Sjogren's disease, idiopathic pulmonary fibrosis and others), sarcoidosis, hypersensitivity pneumonitis, lung cancer, cystic fibrosis, respiratory infections (community-acquired and ventilator-associated pneumonia, pneumocystis pneumonia, tuberculosis, pulmonary infections with non-tuberculous mycobacteria), surveillance after lung transplantation and in immunocompromised hosts [21-25]

Fiberoptic bronchoscopy involves local anesthesia of the nose and oropharynx, the larynx, and the trachea and proximal bronchi. A thin, flexible fiberoptic bronchoscope containing fibers and lens to conduct light and transmit images of the airways is inserted into the trachea and lower airways via the nares or mouth. The bronchoscope also contains a working channel to suction and remove secretions, administer local anesthetic drugs or normal saline solution, and collect bronchial washings, lavage and other samples. Healthy participants typically tolerate bronchoscopy well with only local anesthesia in contrast to clinical bronchoscopy that is usually done under conscious sedation with short acting benzodiazepines or opiate analgesics. Bronchoscopists are assisted by trained nurses who assist in preparing and monitoring patients before bronchoscopy, collecting samples during bronchoscopy and monitoring post-procedure. Modern standards of care for fiberoptic bronchoscopy include the use of pulse oximetry to measure oxygen saturation and pulse continuously during the procedure to monitor patient safety.

2.2 Rationale

Studies performed by the investigators with colleagues in Uganda established that the household contact design is a systematic approach for studying MTB infection and disease [26-30]. Evaluation of

household contacts allows for efficient identification and follow-up of individuals with different stages of MTB exposure and infection. These groups form the basis for comparing epidemiology, transmission dynamics, genetics and immunology to gain insight about risk factors for infection and progression to disease. Results from prior household contact studies in Kampala, Uganda reveal that at the time of household evaluation, 75% of those 15 years and older are TST positive at baseline. Approximately 2/3 of TST negative household contacts will convert their TST from negative to positive within 3 to 6 months. This high rate of TST conversion in healthy TST negative adults is an indication of recent exposure and infection with MTB. Performing BAL on these individuals after MTB exposure and infection provides a unique opportunity to sample immune responses during control and possibly elimination of MTB in the lung, i.e. a direct window in developing *in vivo* protective immunity in the lung. While most studies of MTB infection and disease have focused on changes in peripheral blood immune responses, little is known about immune responses and the presence of MTB bacilli and their products in the lungs of persons exposed and/or recently infected with MTB.

This knowledge is critical for development of vaccines and biomarkers of protective immunity aimed at preventing MTB infection and progression to active TB. This proposed study is a hypothesis-driven research project that builds on past research on MTB exposure, infection and development of latent MTB infection. It is critical to understand the protective immune responses that develop in the human lung in order to further improve TB vaccine development, identify persons at highest risk for progression from latent MTB infection to active disease and optimize TB treatment to reduce treatment failure and relapse. All these events in the natural history of MTB infection and disease start and end in the lung. Knowing which markers in peripheral blood correlate with protective immune responses in the lung during acute MTB infection would be a huge step forward for developing new TB vaccines, diagnostics and treatments.

2.3 Study Objectives

The goals and objectives of this observational study are:

1. To define innate and adaptive immune responses to MTB in the lung following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive tuberculin skin test (TST) and/or positive Interferon-gamma release assay (IGRA) (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI).
2. To define innate and adaptive immune responses in the peripheral blood following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.
3. To compare the rate of positive MTB culture in BAL specimens of healthy adult household contacts of persons with active pulmonary TB of those with a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.
4. To determine if MTB molecules (e.g. proteins, glycolipids, lipids, DNA) can be detected in BAL of healthy adult household contacts of persons with active pulmonary TB who have a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) and in those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.

A better understanding of immune responses to MTB in the lung after MTB infection and exposure is critical for development of vaccines and biomarker of protective immunity and other interventions aimed at preventing MTB infection and progression to active TB.

2.4 Potential Risks and Benefits

2.4.1 Potential Risks

2.4.1.1 Bronchoscopy and Bronchoalveolar Lavage

Flexible fiberoptic bronchoscopy and BAL in Clinical Chest Medicine

Fiberoptic bronchoscopy and BAL have been performed since the 1960s [31] in patients with a variety of pulmonary diseases and in healthy volunteers [32]. Several recent relatively large studies done in the US and Europe have demonstrated the safety of bronchoscopy in people with chronic lung disease, including asthma, COPD and pulmonary hypertension [18-20, 33-36]. Complications of clinical fiberoptic bronchoscopy are infrequent and include cough, bronchospasm, pneumothorax, hemoptysis (usually when brushings, biopsies or transbronchial needle aspiration is done), cardiac arrhythmia, sore throat, nasal trauma, and side effects of local anesthesia (allergic reactions or seizure) or conscious sedation (respiratory depression). Published studies report major complication rates of 0.08 to 0.8% and a mortality rate of 0.01 to 0.04% [37-41]. In a large clinical bronchoscopy study of complications occurring in 20,986 bronchoscopies, hemoptysis of more than 50 ml occurred in 0.26% of patients, mild hemoptysis occurred in 0.19%, hypoxemia in 0.12%, pneumothorax in 0.1%, pulmonary edema in 0.07%, bronchospasm in 0.04%, dyspnea in 0.03% and arrhythmia in 0.02% of patients [42]. Pulmonary infection related to bronchoscopy was rare. Four deaths (0.02%) were reported due to cardiac arrest, pulmonary edema, delayed respiratory failure and shock; all occurred in patients undergoing bronchoscopy with laser treatment.

Fiberoptic Bronchoscopy in Lung Research in Patients and Healthy Volunteers

In addition to clinical diagnosis and treatment, fiberoptic bronchoscopy has been widely and safely used for research in patients with pulmonary disease and healthy human volunteers. Research uses include studies of lung function and immune defenses in normal participants, the pathophysiology of lung inflammation in interstitial lung disease and asthma, evaluation of new diagnostic tests, severity of disease and biologic markers of activity and progression of lung diseases. BAL is performed by advancing the tip of the bronchoscope into a segmental or subsegmental bronchus (see Fig. 1 below), slowly instilling small aliquots of sterile normal saline solution through the bronchoscope, and then gently aspirating the instilled saline to sample cells and inflammatory mediators from the distal bronchi and alveolar space. BAL was introduced in the early 1970s and has provided valuable information about many pulmonary diseases. Transient lowering of oxygen saturation and self-limited fever occurring several hours after lavage are the most frequent side effects.

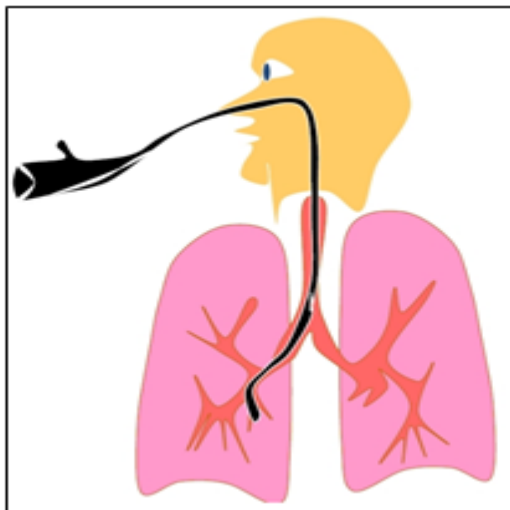


Figure 1: The fiberoptic bronchoscope is passed through the naris, posterior pharynx, larynx and vocal cords. It is then advanced into the lower airway after instilling additional 1 to 2 ml aliquots of topical 1% lignocaine (lidocaine) into the trachea, mainstem, lobar and segmental bronchi via the bronchoscope for local anesthesia. The tip of the bronchoscope is finally gently wedged into a segmental or sub-segmental bronchus of approximately the same caliber as the bronchoscope. Small 30 ml aliquots of sterile physiologic normal saline solution are then slowly aspirated back into a syringe and collected to be taken to the laboratory for studies. Usually 60 to 80% of the instilled saline is recovered; the rest is rapidly absorbed by the lung. The bronchoscope is removed after the bronchoalveolar lavage has been completed. The participant is usually observed for 1-2 hours after bronchoscopy (longer if deemed necessary by the bronchoscopist or study physician). The participant's pulse and oxygen saturation are continuously monitored by non-invasive

pulse oximetry during the bronchoscopy and post-procedure period.

Post-bronchoscopy fever is usually mild and has been associated with higher instilled volumes of normal saline for lavage [43, 44]. The death of a healthy volunteer in 1996 following a research

bronchoscopy heightened concerns about the maximum dose of lidocaine (also called lignocaine) for aerosol and topical anesthesia of the airways during bronchoscopy [33]. Review of this death indicated that several times the recommended maximum dose of lidocaine had likely been administered during the bronchoscopy prompting increased measures to monitor and limit the dose of lidocaine administered during research bronchoscopies. Guidelines of the European Thoracic Society [45], which are also endorsed by American Thoracic Society and the American College of Chest Physicians, recommend that administering up to 8.2 mg of lidocaine per kg of body weight appears safe. Recently reported 2013 guidelines of the British Thoracic Society are consistent with the European and U.S. guidelines [46]. As an additional safeguard in this study, the amount of lidocaine administered to healthy study participants will be limited to 7 mg/kg of body weight, with a maximum dose of 400 mg during the entire procedure.

Clinical and Research Fiberoptic Bronchoscopy in sub-Saharan Africa and Mulago Hospital

Fiberoptic bronchoscopy has been used safely over the past several decades for clinical care and research in resource-constrained countries [47-52], including countries in sub-Saharan Africa and at Mulago Hospital in Uganda. Many of the studies used fiberoptic bronchoscopy to assess the etiology and improve the diagnosis of pulmonary infections in HIV-infected patients. From 1999 to 2000, Dr. Worodria performed bronchoscopy and BAL on 83 HIV-positive adults who were sputum AFB smear-negative [53]. Thirty-nine percent were found to have pneumocystis pneumonia, 24% had TB, 11% had pulmonary Kaposi's sarcoma and one-third had no etiological diagnosis made. Hypoxemic patients on supplemental oxygen, patients with underlying asthma or COPD and severely debilitated patients were excluded from this study. No clinically significant complications, including death, occurred due to the bronchoscopy. Other bronchoscopy studies conducted in Uganda have contributed important knowledge about TB, cryptococcosis and other pulmonary infections with acceptable patient safety. In 2012, fiberoptic bronchoscopy with BAL was used to further describe the epidemiology of *Pneumocystis jirovecii* colonization in hospitalized Ugandans and to evaluate T cell interferon gamma release assays for TB diagnosis [54-56]. Studies from 2010 to 2012 have investigated the role of non-tuberculous mycobacteria in pulmonary disease, causes of pneumonia in patients hospitalized in Kampala, and the frequency of recovering *Cryptococcus neoformans* from BAL fluid [57-59].

A recent study from Malawi reported the results of a detailed audit and interviews of the acceptability and adverse events occurring in 263 research fiberoptic bronchoscopies in 81 healthy HIV-seropositive and seronegative volunteers in studies of pulmonary defenses against pneumonia and TB [60]. The picture below from this paper shows a research bronchoscopy and BAL procedure in a healthy participant in Malawi in progress.

During this study, 19 healthy participants reported any adverse symptoms during or after fiberoptic bronchoscopy. Cough and minor chest pain were reported most frequently. Symptoms were self-limited and resolved within a median of 2 days (range 1 to 7 days), there were no deaths [60].



Figure 2: Bronchoalveolar lavage during a research bronchoscopy in Malawi. The fiberoptic bronchoscope has been inserted into the right nostril of the volunteer and is held in a sub-segmental bronchus of the right middle lobe. The bronchoscopist maintains the bronchoscope position by observing the video monitor (not shown) while the assistant first instills and then aspirates warm sterile saline using gentle hand suction. Informed consent from the volunteer was obtained for the publication of this figure.

Six participants re-attended clinic for their symptoms but none reported that their symptoms would cause them not to decide to undergo another research bronchoscopy. The investigators concluded that research fiberoptic bronchoscopy was acceptable to healthy volunteers and had a low risk of side effects.

In summary, the available evidence suggests that fiberoptic bronchoscopy and BAL can be performed for clinical research in healthy volunteers under careful monitoring by trained physicians and nurses with acceptable comfort and minimal complications and does not pose undue risk to healthy participants. Research bronchoscopy has an acceptable safety record over the past 30 years in multiple countries, including countries in sub-Saharan Africa, and has provided valuable information regarding the diagnosis, treatment and pathogenesis of lung diseases.

Measures to Protect Participant Safety During this Study

To protect participants in this study, participants will undergo medical history and physical examination during screening. Healthy adults between 18 to 50 years of age will be recruited to minimize complications and risks due to age. Adults with asthma and other chronic medical conditions will be ineligible to undergo research bronchoscopy. The total dose of lidocaine used for local airway anesthesia will be limited to no greater than 400 mg. Participants will be monitored by continuous pulse oximetry during bronchoscopy and observed for a minimum of 2 hours post-procedure, or longer if necessary based on the clinical judgement of the bronchoscopist or study physician. Participants with oxygen saturation below 90% at the end of the 2-hour observation period will be admitted for further treatment and observation overnight.

2.4.1.2 Blood Draws

Inserting a needle through the skin to draw blood is mildly and briefly painful. For most people, needle punctures do not cause any serious problems. Infrequently, phlebotomy causes bleeding, bruising, discomfort, infections, dizziness, or fainting. If the participant prefers to draw only partial volume of the blood at a study visit, the full blood volume may be drawn at two different clinic visits within a 3 day period of the study visit date.

2.4.1.3 Chest X-rays

A standard postero-anterior chest radiograph will be taken at the standard 1.8 m distance that exposes participants to a small amount of radiation. The health risk of exposure to small doses of ionizing radiation from indicated medical diagnostic procedures such as chest radiography is

small and generally known to medical professionals and the public. Such small doses of radiation may be potentially harmful, but the risks are so small that they are difficult to measure.

2.4.1.4 Confidentiality

Study-related information will be kept confidential except in the circumstances as detailed. Information concerning the participant, the participant's identity, medical history and information collected during this study may be disclosed if required by law. Such information may also be disclosed or used by others involved in or overseeing the study, such as the independent safety monitor, the study sponsor and its agents, as well as U.S., governmental, regulatory and accrediting agencies. Also, records may be reviewed by the following groups, as applicable to the research:

1. Office for Human Research Protections or other federal, state or international regulatory agencies
2. Uganda Makerere University School of Biomedical Sciences Higher Degrees Research and Ethics Committee
3. Ugandan National Council for Science and Technology
4. University Hospitals Cleveland Medical Center (UHCMC) IRB
5. Case Western Reserve University

2.4.1.5 Lidocaine Administration

Common side effects of lidocaine administration include lightheadedness, dizziness, anxiety, vomiting, local erythema, local edema and abnormal sensations. Serious infrequent adverse reactions include anaphylaxis, seizure, arrhythmia, respiratory depression and cardiac arrest. Lidocaine administration will be limited to a maximum of 7 mg/kg of bodyweight, and not to exceed 400 mg. This dosage is considered safe by the British Thoracic Society and European Thoracic Society [45, 46] and is endorsed by the American Thoracic Society and the American College of Chest Physicians.

2.4.2 Potential Benefits

Participants may benefit from the increased monitoring and health care exams they will receive as part of the study. This monitoring may result in more timely referrals to health care services for treatment of illnesses beyond what is being specifically screened for in this study. Health checks will include assessment of vital signs and a physical exam and additional workup as clinically indicated. There is no direct benefit otherwise for participants undergoing bronchoscopy.

3.0 OBJECTIVES

3.1 Hypothesis and Objectives

Hypothesis: There are innate and adaptive immune responses to MTB that are unique to the lung, that are measurably different in people with MTB infection (LTBI or CNVT) compared to people who are exposed to MTB but resist infection (RLTBI), and are not equivalent to those detected in peripheral blood.

Objectives:

1. To define innate and adaptive immune responses to MTB in the lung following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive tuberculin skin test (TST) and/or positive Interferon-Gamma Release Assay (IGRA) (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI).
2. To define innate and adaptive immune responses in the peripheral blood following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those with a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.

3. To compare the rate of positive MTB culture in BAL specimens of healthy adult household contacts of persons with active pulmonary TB of those with a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) to those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.

4. To determine if MTB molecules (e.g. proteins, glycolipids, lipids, DNA) can be detected in BAL of healthy adult household contacts of persons with active pulmonary TB who have a positive TST and/or positive IGRA (i.e. persons with LTBI or CNVT) and in those who are TST and IGRA negative (i.e. persons with RLTBI) in parallel with Objective 1.

4.0 STUDY DESIGN

This is a cross-sectional observational study of HIV sero-negative adults to assess host immunological responses in the lungs and peripheral blood of adults following exposure to patients with culture-confirmed or GeneXpert positive pulmonary TB. The study will enroll persons identified from the community in Kampala, Uganda. The goal is to gather research data by collecting specimens to help define the innate and adaptive immune responses to MTB in the lung and blood following MTB exposure. This is done by comparing adults who are TST and IGRA negative (RLTBI) to people who are TST and/or IGRA positive (LTBI or CNVT). Adult TB household contacts who may meet eligibility criteria for this bronchoscopic evaluation will have been identified through separate IRB-approved TB household contact studies. These two studies are 1) The Kawempe Community Health Study and 2) Resistance to Latent MTB Study (DMID protocol 15-0093). Individuals interested in participating will be screened and those meeting eligibility criteria will be placed into three groups –

- 1) IGRA **and** TST positive HHCs (LTBI)
- 2) TST **and** IGRA negative HHCs (RLTBI)
- 3) HHCs, who recently (within 6 months) converted their IGRA from negative to positive (i.e. IGRA converters (CNVT)).

Twenty-five (25) participants will be enrolled into each study group. All enrolled participants will complete baseline assessments, as well as pre-bronchoscopy, bronchoscopy, and post bronchoscopy visits.

Two Study Phases to Ensure Safety for Participants Undergoing Bronchoscopy

To ensure the safety and well-being of participants undergoing bronchoscopy and BAL, we will initially enroll 5 participants for bronchoscopy and BAL. After completion of bronchoscopy and BAL on these first 5 participants, enrollment will be halted until safety data has been reviewed by the sponsor. A local independent physician safety monitor (ISM) will be appointed to review the bronchoscopy safety data. The ISM will review the participants' pre-bronchoscopy, bronchoscopy and follow-up notes, including their laboratory test results, vital signs and oxygen saturation, the total dose of lidocaine administered under topical anesthesia for the bronchoscopy, the amount of sterile normal saline instilled and percent returned from the BAL, and any adverse experiences occurring during and after bronchoscopy. After review, the ISM will submit a written report to the investigators summarizing his/her review and any recommendations. This report will be provided to the study sponsor and the responsible IRBs. After review of the report, the sponsor will contact the investigators regarding permission to resume enrollment. Subsequent safety review by two local ISMs is described in further detail in protocol Section 9.5. The ISMs will continue to review bronchoscopy safety data after each additional 10 participants have undergone bronchoscopy and report to the investigators and IRB but enrollment will not be halted unless any of the study stopping rules listed in Section 9.4 are met.

5.0 STUDY POPULATION

Household contacts (HHCs) of individuals who have a confirmed diagnosis of active TB and who meet eligibility criteria will be enrolled in this study. Only household contacts age 18 years to 50 years of age who are not acutely ill with TB or other serious illness/infection after a health check will be screened for enrollment. Seventy-five (75) total HHCs who demonstrate no sign of

TB disease and who are HIV seronegative will be consented and enrolled, as described below.

- 25 who are TST **and** IGRA negative (RLTBI),
- 25 who are TST **and** IGRA positive (LTBI), and
- 25 who are TST and IGRA negative who converted to IGRA positive and remained asymptomatic within 6 months (CNVT) prior to screening for this study

Enrolled participants that do not complete the bronchoscopy procedure or have insufficient samples from the procedure for evaluation will be replaced.

5.1 Selection of the Study Population

Participants for this study will be recruited from two ongoing household contact studies conducted by the principal investigators. These two ongoing studies are 1) The Kawempe Community Health Study and 2) Resistance to Latent MTB Study (DMID protocol 15-0093). Both ongoing household contact studies require that enrolled HHCs have lived in the same building (house, hut, or apartment), or a portion of the building, as a confirmed TB index case, thereby sharing air-space with the index case, for at least one week during the three-month period immediately preceding the diagnosis of TB in the index case. Household contacts are further screened and not enrolled in the currently ongoing studies if the HHCs are found to have active TB disease or other febrile illness or uncontrolled disease, or if they have a chest radiograph consistent with active TB.

HHCs from the ongoing study cohorts described will initially be assessed from available study data, including age, living arrangements with a TB index case, and initial HIV status. The HHC will then be approached for interest in this bronchoscopy study. Individuals interested in participating will be further assessed through a scheduled screening visit. If consent is obtained and eligibility is confirmed, relevant data from the ongoing HHC studies utilized to assess eligibility will also be used for this study.

5.2 Inclusion and Exclusion Criteria

5.2.1 Enrollment Inclusion Criteria

- Provision of informed consent
- Individuals 18 to 50 years of age
- Individuals having lived in the same building (house, hut, or apartment), or a portion of the building, as the index TB case for at least one week during the 3 months preceding the diagnosis of TB in the index case. This will be obtained from the enrollment records of the Kawempe Community Health Study or DMID Protocol 15-0093
- HIV seronegative individuals
- Complete blood count (CBC), white blood cell (WBC) differential count within the laboratory's normal range
- Serum alanine aminotransferase (ALT) and total bilirubin within the laboratory's normal range
- Prothrombin (PT) and activated partial thromboplastin (aPTT) time and platelet count within the laboratory's normal range
- Negative urine pregnancy test in women of child-bearing potential
- Healthy, non-smoking individuals

5.2.2 Enrollment Exclusion Criteria

- Previous episode of TB
- TB suspect or active TB
- Chest radiograph consistent with active TB
- Current use of immunosuppressive agents
- Other febrile illness or uncontrolled disease
- Past history of allergic reaction to lidocaine or other topical anesthetics
- History of cardiac disease
- History chronic renal failure

- History of cancer
- History of complications following bronchoscopy procedure
- Self-reported previously diagnosed hypertension, diabetes, asthma or chronic obstructive pulmonary disease (COPD)
- History of seizure disorders
- HIV-positive individuals
- Pregnant or breast-feeding/lactating women

Subjects whose screening lab values fall outside of the laboratory normal ranges may be re-screened, or may be enrolled if the values are documented to be not clinically significant as assessed by the study investigator, study coordinator or his/her physician designee, all of whom are medically trained physicians.

6.0 STUDY PROCEDURES AND EVALUATIONS

All consenting participants may have initial/baseline test results available as part of their participation in the two ongoing household contact studies conducted by the investigators. As indicated in the study Time and Events schedule (Appendix A), results from these HHC studies for chest radiograph, if completed within the previous 6 months, may be used in assessing eligibility for this bronchoscopy study. Abstraction of IGRA result from ongoing household contact studies will be used for bronchoscopy study screening. Additional procedures detailed in Appendix A will be performed to assess final eligibility for the study.

6.1 Study Procedures

6.1.1 Clinical Characteristics, Medical History, Targeted Physical Exam, Vital Signs and SpO₂

Data on clinical characteristics of the participants will be obtained through a standard medical history and targeted physical examination by a study medical officer/clinician using a standard data collection form. The form will collect information regarding symptoms and signs of active TB as well as examination of the eyes, oropharynx, neck, heart, lungs, abdomen, and extremities. Height, weight, blood pressure, heart rate, respiratory rate and SpO₂ (oxygen saturation by pulse oximetry) will be measured and recorded.

6.1.2 Blood Collection

Blood will be collected using standard aseptic technique. The study team will draw 4-5 ml for routine clinical laboratory evaluations as part of the screening and pre-bronchoscopy visits. At time of bronchoscopy a few days later, sixty (60) ml will be drawn for research evaluations to be performed in parallel with BAL fluid studies. Sixty (60) ml of blood is required to complete all clinical and immunologic tests during the bronchoscopy visit. Since blood draws will be carried out in healthy individuals, over 18 years of age, with or without TB infection but not active disease, drawing 60 ml is justified and necessary for the research studies. This volume of blood is proportional to the amount usually requested in pediatric studies (1 ml/kg weight). Blood cells and plasma will be used for antibody studies, gene expression, cellular function assays, flow cytometry, DNA and proteomic studies. 60 ml is the maximum amount of blood needed for these studies. We have conducted similar studies previously, and have observed that these assays will not work if there are not enough cells. Note that very little is known about how the human lung responds to exposure to MTB bacteria, so that an array of studies is needed to answer the important public health questions. We have calculated the minimum amount necessary to obtain meaningful results.

6.1.3 Urine Collection

Urine will be collected using standard procedures. All women will have a urine pregnancy test at baseline and within 72 hours before the bronchoscopy. Urine will be tested using a pregnancy dipstick test with the sensitivity level of 25 mIU hCG/mL.

6.1.4 HIV Testing

Counseling prior to and following HIV testing, and reporting of HIV testing results will be done in accordance with Ugandan national guidelines. For study purposes, HIV-1 infection is defined as a positive result using any licensed rapid HIV test or any licensed HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit. An initial rapid HIV finger-stick test will be used in this study. Confirmation of an initial positive test result is required and must utilize a different method than the one used for the initial assessment. The confirmatory test to be used for this study will be plasma HIV RNA viral load. Negative HIV testing results do not require confirmatory testing.

6.1.5 Chest Radiographs

Standard postero-anterior chest radiographs taken at the standard 1.8 m distance will be done in the radiology department used by the Uganda-CWRU Research Collaboration for TB research studies.

6.1.6 Bronchoscopy and BAL Procedure

Research bronchoscopies for this study will be performed in the endoscopy suite at Mulago Hospital, by an experienced clinical bronchoscopist who is a licensed consultant pulmonary physician in Uganda. The Ugandan bronchoscopist will receive additional training in research bronchoscopy and bronchoalveolar lavage methods by a U.S. pulmonary physician before the study begins.

The cleaning, disinfection, storage and maintenance of the research bronchoscope used in the study will be performed by a trained bronchoscopy research nurse.

Research bronchoscopy procedures will be as follows:

- A pre-bronchoscopy assessment will be done no more than 72 hours prior to bronchoscopy.
- Participants will be weighed, have vital signs taken, and routine clinical laboratory evaluations prior to bronchoscopy. Participants with abnormal vital signs or clinical laboratory results will not be allowed to proceed with bronchoscopy.
- Prior to the bronchoscopy, a physician will confirm previously granted informed consent for the procedure from the research participant, review vitals and laboratory values, and perform a physical examination. The participant will be offered a chance to ask questions, and may decline participation at this point. Participants who do not have a bronchoscopy performed for any reason will be replaced.
- The participant's medical history will be reviewed and updated, verifying that no contraindicated condition has been diagnosed since the participant was enrolled.
- Participants will only receive local anesthesia with topical lidocaine and lidocaine instilled through the bronchoscope.
- Lidocaine administration will be limited to a maximum of 7 mg/kg of bodyweight, and will not exceed 400 mg in total.
- Participants will be monitored with continuous pulse oximetry throughout the procedure and have vital sign checks at least every half an hour during the observation period following the procedure.
- During bronchoscopy, all participants will routinely receive 2 liters per minute of supplemental oxygen by nasal cannula.
- During the bronchoscopy procedure, 30 ml aliquots of sterile normal saline for intravenous injection will be introduced into the lung segment via the bronchoscope and then gently aspirated to obtain BAL fluid and cells. A total of 240 ml of sterile normal saline solution will be instilled and aspirated typically resulting in 150-200 ml of returned BAL fluid at the end of the procedure for analysis.
- Emergency supplies and a kit with emergency medications will be available at bedside during the bronchoscopy procedure.

- Participants will be monitored for a minimum of 2 hours after completion of bronchoscopy or longer based upon clinical judgment.
- A post bronchoscopy assessment will be done within 3 days following bronchoscopy.

6.1.7 Tuberculin Skin Testing (TST)

For enrolled participants, tuberculin skin testing will have been performed as part of their recruitment from ongoing household contact studies. TST is performed using 5 TU of purified protein derivative (PPD-S, Sanofi Pasteur, Tubersol), placed intradermally in the skin of the non-dominant forearm. The diameter of induration is measured in millimeters between 48 and 72 hours following placement, by trained staff using digital calipers to minimize terminal digit bias. The previously positive (≥ 10 mm) or negative (< 10 mm) results will be recorded for this study, but testing will not be repeated as part of this study.

6.2 Laboratory Evaluations

6.2.1 Specimen Collection, Handling, and Storage

The study team will draw 4-5 ml of venous blood for routine clinical laboratory evaluations as part of the screening and pre-bronchoscopy visits which are a few days before the 60 ml of venous blood obtained at time of bronchoscopy for research evaluations. Blood for routine laboratory testing will be taken to certified laboratories as described below.

Blood for research evaluations will be taken to the Joint Clinical Research Centre (JCRC) Immunology Laboratory in Lubowa, which is managed by the Uganda-CWRU Research Collaboration. This research laboratory is capable of performing a wide range of immunologic testing and sample storage, including 8-color flow cytometry (intra and extracellular), short term *in vitro* cell cultures, magnetic bead cell separation, and measurement of cytokines (IFN- γ , TNF- α , transforming growth factor (TGF)- β , IL-10) by ELISA and ELISPOT assays. This laboratory will be used for IGRA testing, all other immunology laboratory work, and specimen storage. These samples include plasma/serum, PBMC and mRNA in PAXgene tubes.

BAL fluid collected during bronchoscopy will also be transported to the JCRC Immunology and JCRC TB/Mycobacteriology Laboratory (also managed by the Uganda-CWRU Research Collaboration) for processing, immunologic and microbiologic analysis, and storage.

Residual specimens of blood and BAL fluid will be stored at the JCRC Immunology Laboratory in Uganda for shipment to Case Western Reserve University or other laboratories for additional testing. During initial consent for this study, participants will indicate on a separate specimen storage consent form if they agree to allow residual linked samples to remain stored indefinitely for future research, or if they consent to have their residual linked samples delinked (anonymized) and stored indefinitely for future research. These paired peripheral blood and BAL specimens will be very valuable for immediate and future use. To our knowledge, no other investigators have defined as carefully as our studies healthy individuals heavily exposed to MTB who have developed MTB infection or resisted MTB infection without developing TB disease. Alternatively, the participant may request that their samples be destroyed at the completion of the study. Participants will be informed that they may decline to have samples stored for future research and still participate in this study. Participant consent will be tracked, recorded, and samples handled accordingly. If future research studies are done using residually stored specimens, IRB approval will be obtained before any additional research is conducted.

6.2.2 Laboratory Procedures

6.2.2.1 Routine Laboratory Measurements

The following routine clinical laboratory tests will be performed at the Infectious Disease Institute

(IDI) Core Laboratory, a College of American Pathologists (CAP) certified laboratory, located on the Mulago Hospital campus during the study as indicated in the Time and Events Schedule (Appendix A). Tests not available at the IDI Core Laboratory such as the PT/aPTT will be performed by other certified laboratories. Methods used in performing these tests are described in a manual of procedures maintained at the laboratory. These laboratory measures will include:

- HIV confirmatory plasma HIV RNA viral load test for HIV+ participants (from rapid HIV test as indicated)
- Confirmatory urine pregnancy testing for women of childbearing potential if the rapid test done in the clinic is positive
- Complete blood count (CBC) with differential WBC and platelet counts
- Serum alanine aminotransferase (ALT) and total bilirubin
- PT/aPTT

6.2.2.2 Interferon-gamma Release Assay (IGRA)

Interferon-gamma release assays (IGRA) will be conducted at the JCRC Immunology Laboratory. These assays will be batched and run routinely. IGRA test results from ongoing household contact studies will be abstracted at screening for the purposes of identifying screening cohort within the bronchoscopy study. After enrollment, IGRA will be performed as described in Appendix A. We will use the QuantiFERON test as IGRA for this study. Whole blood will be incubated with test antigens for 16 to 24 hours. The antigens are synthetic MTB peptides and mitogen phytohemagglutinin (PHA) serves as positive control for the assay). Saline (nil sample) serves as negative control and provides the IFN-gamma background levels. After incubation, IFN-gamma levels are determined by ELISA. The amount of MTB specific IFN-gamma released is determined by subtracting the amount in the nil sample well from the amount in MTB antigen stimulated wells and if above 0.35 Units/ml, the test is positive. IGRA provides a binary (positive/negative) readout.

6.2.2.3 Immune Function Assays

Blood, serum, PBMC, BAL fluid and cells will be collected and used for assays to measure innate and adaptive immune and other host responses to MTB in peripheral blood and alveolar spaces. Some assays will be performed in real time and others on batches of frozen (in liquid nitrogen or at -80°C) samples. The Collaboration's labs in Uganda perform a number of immunologic and microbiologic assays at excellent QA/QC standards that include, in addition to preparation of serum/plasma, peripheral blood mononuclear cells (PBMC), RNA and DNA extraction, T cell ELISPOT assays, cytokine ELISAs, multi-parameter flow cytometry, purification of specific immune cell populations (e.g. CD4+ T cells, monocytes/macrophages) and functional assays such as whole blood mycobacterial killing and stimulation assays. As much as possible, assays will be performed in Uganda, and the Collaboration will continue to transfer technology and assays to Uganda. More technically advanced assays not available in Uganda will require shipment to the US. These include host cell gene expression (Array and RNA seq), proteomic and metabolomics assays. Multi-parameter flow cytometry is used to measure cell surface molecules and intracellular cytokines on T cells, macrophages and other innate immune cells, which characterize specific cell types, their abundance and functional activation status. ELISPOT and ELISA are used to measure MTB-specific CD4+ and CD8+ T cell responses and measure the frequency of antigen-specific T cells. Proteomics, metabolomics, RNA expression profiling and multiplex cytokine measurements and their analyses are part of the systems biology approaches to understanding complex host responses in health and disease. The overall goal of these assays is to maximize the amount of new information that can be learned from these precious samples in order to understand the host response to MTB in the lung in MTB infection and resistance to MTB infection, and to identify host markers that reflect host responses in the lung but are measureable in peripheral blood.

6.2.2.4 BAL Fluid Analysis

BAL fluid collected from the BAL procedure will be evaluated for the following immunological, microbiological, and gene expression parameters. Some assays will be performed in real time while others will be performed on stored frozen (liquid nitrogen and -80°C) samples:

Immunologic and Other Host Response Parameters:

1. mRNA expression by BAL cells
2. Cytokine protein expression, proteomics and metabolomics on BAL fluid. Phenotypic analysis of cell surface and intra-cellular cytokine molecules of innate and adaptive immune BAL cells by flow cytometry
3. T cell responses to MTB antigens by ELISPOT and ELISA

Microbiological Parameters:

1. Presence of viable MTB by culture
2. Presence of MTB proteins, nucleotide and (glyco-) lipids by ELISA, PCR and mass spectrometry (MassSpec)

PCR and mass spectrometry for MTB nucleotide, proteins and (glyco-) lipids and other molecular methods will be used not only as ancillary methods in the identification of MTB, but also to determine if more sensitive means can detect MTB molecules in BAL fluid and if they are indicative of MTB infection.

7.0 STUDY SCHEDULE

See the Time and Events Schedule (Appendix A) for details of time points and assessments to be performed for enrolled participants. All participants will be evaluated at a screening and baseline visit for stratification into the LTBI, CNVT, and RLTBI groups. All will have screening, baseline and three subsequent study visits – a pre-bronchoscopy visit scheduled 72 hours prior to the bronchoscopy procedure, the bronchoscopy procedural visit, and a post-bronchoscopy visit scheduled 72 hours maximum following the procedure. Procedures for each visit are described in this section.

7.1 Initial Screening Visit

All participants will undergo the following evaluations during the initial screening visit to assess eligibility for the study.

- Counseling and consent for bronchoscopy with bronchoalveolar lavage and HIV testing
- Demographic information, medical history and targeted physical examination, vital signs and and SpO2
- Chest radiograph (if not performed during the preceding 6 months with a written report available for review)
- Rapid HIV testing using approved HIV rapid test kits
- Blood draw for the following:
 - HIV confirmatory test if HIV+ from rapid test
 - Complete blood count with platelet count
 - Serum alanine aminotransferase (ALT), total bilirubin and serum creatinine
 - PT and aPTT
 - IGRA result abstraction from ongoing household contact studies to assess study eligibility and to determine cohort
- Urine pregnancy test for women of childbearing potential

7.2 Baseline Eligibility Assessment

After review of results from screening evaluations, including history of IGRA and TST test results, eligible participants will be contacted regarding their eligibility for the study. Participants may come to the clinic if they prefer, but a study visit is not required if participants can be reached by telephone. Participants with concordant IGRA and TST results (i.e. IGRA and TST both positive for LTBI or IGRA and TST both negative for RLTBI), or participants whose clinical history documents recent conversion from concordant IGRA and TST negative to IGRA positive (i.e. converters (CNVT)) will be asked to continue with the bronchoscopy study. If they agree to continue, the three bronchoscopy study visits will be scheduled and confirmed with the participant.

7.3 Pre-Bronchoscopy Visit

A clinic visit will be scheduled no later than 72 hours prior to bronchoscopy. During this pre-bronchoscopy visit, the following evaluations will be performed:

- Counseling and confirmation of consent for bronchoscopy and bronchoalveolar lavage
- Medical history, targeted physical examination, vital signs and SpO2
- Blood draw for the following:
 - Complete blood count with platelet count
 - PT and aPTT
- Urine pregnancy test (for women of child-bearing potential)

The participant's total WBC count, hemoglobin, platelet count, and PT and aPTT must be within the local laboratory's normal range, and female participants must have a negative urine pregnancy test before proceeding with bronchoscopy. Subjects whose pre-bronchoscopy lab values fall outside of the laboratory normal ranges may have labs repeated, or may be enrolled

if the values are documented to be not clinically significant as assessed by the study investigator, study coordinator, or his/her physician designee, all of whom are medically trained physicians.

The participant's vital signs and oxygen saturation on room air must be within normal limits (oral or tympanic membrane temperature 36.5-37.3°C, pulse 60-100 beats per minute, respiratory rate 12-20 breaths per minute, blood pressure 90/60 – 120/80 mm Hg, oxygen saturation by pulse oximetry (SpO₂) 95-100% on room air) before proceeding with bronchoscopy. Participants whose vital signs fall outside normal limits may continue if assessed as not clinically significant by the study investigator, study coordinator, or his/her physician designee prior to the procedure, all of whom are medically trained physicians.

Participants will be given written instructions for their bronchoscopy scheduled visit, including that the participant must be nil per os (NPO), or can have nothing by mouth (no food or water) for 6 hours prior to the procedure. The participant will also be given an information sheet providing details concerning the procedure itself, including illustrations of equipment to be used and what to expect during and after the procedure.

7.4 Bronchoscopy Visit

Bronchoscopy will be done no later than 72 hours following the pre-bronchoscopy visit. The following will be performed at this visit:

- Additional counseling and confirmation of consent for bronchoscopy and bronchoalveolar lavage
- Clinical history including time of last oral intake and targeted physical examination, clinical history, vital signs and SpO₂
- Blood draw (60 ml) for immunologic testing, and PBMC and serum/plasma storage
 - IGRA testing (for LTBI, CNVT, and RLTBI cohorts)
- Bronchoscopy and BAL

The participant's vital signs and oxygen saturation on room air must be within normal limits (oral or tympanic membrane temperature 36.5-37.3°C, pulse 60-100 per minute, respiratory rate 12-20 breaths per minute, blood pressure 90/60 – 120/80 mm Hg, oxygen saturation by pulse oximetry (SpO₂) 95-100% on room air) before proceeding with bronchoscopy and BAL. Participants whose vital signs fall outside normal limits may continue if assessed as not clinically significant by the bronchoscopist prior to the procedure. Participants who are wheezing or who have arrhythmias or abnormal heart sounds on physical examination will not undergo bronchoscopy.

7.5 Post-Bronchoscopy Visit

A return clinic appointment will be scheduled for participants within 3 days following the bronchoscopy procedure. This is the final visit scheduled for the bronchoscopy study.

The following evaluations will be performed:

- Targeted physical examination, clinical history, vital signs and SpO₂
- Additional counseling as indicated

7.6 Criteria for Discontinuation or Withdrawal of a Participant from the Study

The investigator may discontinue or withdraw participants from the study as warranted for any of, but not limited to the following reasons:

- The participant has a medical condition for which continued participation or completion of study procedure(s), in the opinion of the investigator, would pose a risk to the participant or would be likely to confound interpretation of the results
- The participant withdraws consent
- The participant is lost to follow-up
- The participant develops a respiratory or other febrile illness,
- The participant converts from a negative to positive pregnancy test while on study
- The study is terminated

Participants may withdraw voluntarily from participation at any time. Participants who withdraw consent will be referred to appropriate clinics for medical care if indicated.

If a participant experiences any adverse event or indicates by hand signal to study staff that they wish to halt bronchoscopy, the procedure will be halted. In such circumstances, the participant will still be scheduled for the post-bronchoscopy visit for final safety and clinical follow-up.

Participants who withdraw from the study at any time prior to the bronchoscopy being performed, or have insufficient samples collected from BAL for evaluation, or are removed by the investigator prior to bronchoscopy as described above, will be replaced.

8.0 ASSESSMENT OF OUTCOME MEASURES

BAL specimens will undergo microbiologic and immunologic testing. Immune function studies will also be performed on peripheral blood immune cells to compare peripheral with lung immune responses. These laboratory objectives will be correlated with the clinical outcome measures described below.

1. To use bronchoscopy to further define innate and adaptive immune responses to MTB in the lung following MTB exposure in healthy adult household contacts of persons with active pulmonary TB by comparing lung immune responses of those who are TST and/or IGRA positive, i.e. infected, LTBI or CNVT, and those who remain TST and IGRA negative (i.e. exposed but not infected, RLTI). For this objective, immune function assays with BAL and peripheral blood cells will be performed using flow cytometry to measure cell surface markers on T cells and macrophages and other innate immune cells, ELISPOT to measure antigen specific T cell responses and ELISA to measure cytokine and chemokine proteins. We will also measure host mRNA and metabolic profiles in BAL and peripheral blood specimens. Most assays will be performed in the TBRU immunology lab at the JCRC in Kampala. For some tests, samples will be sent to the US for advanced testing, i.e. metabolomics, proteomics and mRNA profiling (see section 6.2.2.3 for details).

2. To compare innate and adaptive immune responses in the peripheral blood of healthy adult household contacts of persons with active pulmonary TB, who are TST and/or IGRA positive, i.e. with LTBI or CNVT, and those who remain TST and IGRA negative (i.e. exposed but not infected, RLTI) to the lung immune responses described in the above paragraph.

3. To compare the rate of positive MTB culture in BAL specimens of healthy adult household contacts of persons with active pulmonary TB who are TST and/or IGRA positive, i.e. with LTBI or CNVT, and those who remain TST and IGRA negative (i.e. exposed but not infected, RLTI). BAL specimens will be cultured on solid and liquid media in the TBRU mycobacteriology lab at the JCRC in Kampala.

4. To determine if MTB molecules (e.g. proteins, glycolipids, lipids, DNA) can be detected in BAL of healthy adult household contacts of persons with active pulmonary TB who are TST and/or IGRA

positive, i.e. with LTBI or CNVT, and those who remain TST and IGRA negative (i.e. exposed but not infected, RLTI) and used to differentiate these 3 clinical groups of contacts.

9.0 SAFETY ASSESSMENT AND REPORTING

9.1 Definition of Adverse Event (AE)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participant undergoing a study procedure regardless of its causal relationship to the study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally related to the study procedure. The occurrence of an AE 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.

AEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include a Physician, Nurse Practitioner or Dentist) 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 or stabilization.

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

Medical conditions pre-existing to bronchoscopy but that do not affect eligibility will be recorded in the participant's medical history and will not be considered an adverse event unless the condition deteriorates at any time during the study.

Severity of Event

All AEs will be assessed by the clinician using the 2007 DMID Adult Toxicity Tables. For events not included in the tables, the following guidelines will be used to quantify intensity.

- 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 incapacitating.
- Life threatening: any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Severity of adverse events deemed related to the bronchoscopy will further be graded as follows:

GRADE 1	Mild	Transient or mild discomfort related to bronchoscopy (<72 hours); no/minimal medical intervention/therapy required; does not interfere with day to day activities
GRADE 2	Moderate	Mild to moderate limitation in activity related to bronchoscopy (<72 hours); some assistance may be needed; no or minimal medical intervention/therapy required; may interfere with some normal daily activities
GRADE 3	Severe	Marked limitation in activity related to bronchoscopy; some assistance usually required; medical intervention/therapy required, hospitalizations possible; events interrupt normal daily activities
GRADE 4	Life-Threatening	Extreme limitation in activity related to bronchoscopy; significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable; might result in death
GRADE 5	Death	

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

The clinician's assessment of an AE's relationship to bronchoscopy is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to bronchoscopy assessed by the bronchoscopist or subsequently by the investigators using the terms: related or not related. The following guidelines will be used.

Related – There is a reasonable possibility that the study procedure caused the adverse event.

Reasonable possibility means that there is evidence to suggest a causal relationship between the study procedure and the adverse event.

Not Related – There is no reasonable possibility that the study procedure caused the event.

AEs deemed related to bronchoscopy will be classified as minor or major for reporting and halting using the following criteria.

Minor adverse events related to bronchoscopy include transient coughing and/or oxygen desaturation during bronchoscopy that resolve without specific intervention, and post-procedure symptoms consistent with bacterial bronchitis that respond appropriately to antibiotic treatment. Bacterial bronchitis will be the presumed diagnosis for any participant who developed productive cough that did not spontaneously resolve within 24 hours following bronchoscopy, and who did not report other symptoms suggestive of pneumonia. Participants who develop symptoms consistent with bacterial bronchitis will be treated with oral antibiotics and would be expected to have significant improvement in their symptoms with 48 to 72 hours.

Major adverse events related to bronchoscopy include events that require specific treatment in the bronchoscopy suite and recovery area, or transfer to another hospital location or admission for additional monitoring and/or treatment. Major AEs include significant respiratory decompensation during the procedure or evidence of severe or progressive lidocaine toxicity or death. Major events will also include severe wheezing requiring repeated bronchodilator administration, epistaxis requiring nasal packing, seizure, anaphylaxis or severe allergic

reaction, hemoptysis of greater than blood streaked sputum, pneumothorax requiring aspiration or chest tube placement, pneumonia or cardiac arrhythmia other than sinus tachycardia.

9.2 Definition of Serious Adverse Event (SAE)

An adverse event 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 - an adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an adverse event, which had it occurred in a more severe form, might have caused death.
- in-patient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- important medical events that may not result in death, be life-threatening, or require hospitalizations 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.

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution or stabilization by a study clinician
- reviewed and evaluated by a study clinician

The study clinician will continue to monitor the SAE until satisfactory resolution of the event to baseline or until the participant’s condition is deemed to be chronic or stabilization of signs and symptoms or discharge from hospital, and grading for severity and relatedness to the study procedures.

9.3 Reporting Procedures

AEs and SAEs will be documented from the first occurrence through the final scheduled study visit.

9.3.1 Adverse Event Reporting

The Investigator is responsible for reporting all AEs that are observed or reported during the study. AEs will be captured in the participant study file on the appropriate case report form. The Study Coordinator will be responsible for completing these forms and sending them to the Coordinating Center who will then notify the IRBs and sponsor, as required. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to bronchoscopy, time of resolution of the symptoms/event, seriousness, need for medical intervention and outcome.

Unexpected, clinically significant abnormal laboratory test values will also be reported as adverse events and will be followed to resolution or stabilization or until no longer deemed clinically significant by the UCRC staff Medical Officer.

All AEs will be summarized and reported to the sponsor, and the ISM (and back up ISM) on a monthly basis or at another interval deemed appropriate by the sponsor. AEs will be reported to the IRBs at least annually, and according to their reporting requirements.

9.3.2 Serious Adverse Event Reporting

The Investigator is responsible for reporting all SAEs that are observed or reported during the study. SAEs will be captured in the participant study file on the appropriate case report form. All SAEs (serious AEs) must be submitted immediately, within 24 hours of site awareness, to the sponsor or their designated representative for this study. Other supporting documentation of the event may be requested by the sponsor and should be provided as soon as possible.

The process following immediate reporting of SAEs (within 24 hours of site awareness described above) will follow the same steps as AE reporting.

9.3.3 Detection of Adverse Events and Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

If any of the following are detected during or within 72 hours of the bronchoscopy procedure, it will be considered to be an adverse event:

- a) Fever
- b) Chills
- c) Chest pain
- d) Wheezing
- e) Shortness of breath
- f) Bacterial bronchitis
- g) Hemoptysis
- h) Oxygen desaturation (defined as below 90% for more than 1 minute)
- i) Productive cough
- j) Allergic reaction to lidocaine
- k) Any other significant untoward medical occurrence, as defined by ICH Good Clinical Practice guidelines

When a participant presents with an abnormal laboratory test value, abnormal clinical finding or adverse event, the clinician will evaluate and treat the participant as clinically indicated.

9.3.4 Type and Duration of the Follow-up of Participants After Adverse Events

Participants will be monitored for AEs and SAEs as stated above. Participants who experience any type of adverse event will be monitored, inclusive of using pulse-oximetry, and if clinically indicated, will be treated using supplemental oxygen or other such treatment as judged necessary by the clinician. The participant will be followed-up until satisfactory resolution of the event to baseline or until the participant's condition is deemed by the clinician to be chronic or stabilization of signs and symptoms or discharge from hospital.

9.4 Halting Rules

As stated earlier, after completion of bronchoscopy and BAL on the first 5 participants, enrollment will be halted until their safety data has been reviewed by the sponsor. A local independent physician safety monitor (ISM) will be appointed to assist in this review of the bronchoscopy safety data and provide a written report to the investigators, principal investigator (PI), study sponsor, and the responsible IRBs. After review of the report, the sponsor will contact the investigators regarding permission to resume study enrollment.

Two local ISMs will continue to review the bronchoscopy safety data after each additional 10 participants have undergone bronchoscopy and BAL and report to the investigators, PI(s), and IRB as described in Section 9.5 but enrollment will not be halted unless any of the study stopping rules listed below are met. The ISM report will be provided to the study sponsor upon request.

The study will be halted if any major adverse event related to bronchoscopy as defined in section 9.1 occurs. The study may also be halted for assessment of relatedness, severity, and/or seriousness of any adverse events by the PI, IRB, or the study sponsor. After review of the event(s), the PI(s) will contact the investigators regarding permission to resume study activities and

any needed corrective actions or amendments to the protocol. The investigator will inform the IRBs of the PI(s') recommendations before resuming study activities.

The study may be terminated prematurely by the PI, IRB, or ISMs in the event that a serious adverse event, or a pattern of non-serious adverse events, is judged (a) causally related to bronchoscopy procedures and (b) jeopardizes the safety of future study participants. In the event the study is terminated prematurely, study participants will be evaluated by the UCRC staff Medical Officer and local PI and referred for clinical care if indicated.

9.5 Safety Oversight via Independent Safety Monitor

To ensure the safety and well-being of participants undergoing bronchoscopy and BAL, we initially enrolled 5 participants for bronchoscopy and BAL. After completion of bronchoscopy and BAL on these first 5 participants, enrollment was halted until safety data was been reviewed by the sponsor. A local independent physician safety monitor (ISM) with relevant expertise was appointed to assist reviewing bronchoscopy safety data for study participants. A backup local independent physician safety monitor (ISM) will also be identified to assist in reviewing bronchoscopy safety data for study participants in the event that the primary ISM is unavailable. The ISM reviewed the participants' pre-bronchoscopy, bronchoscopy and follow-up notes, including their laboratory test results, vital signs and oxygen saturation, the total dose of lidocaine administered under topical anesthesia for the bronchoscopy, the amount of sterile normal saline instilled and percent returned from the BAL, and any adverse experiences occurring during and after bronchoscopy.

After review, the ISM submitted a written report to the investigators summarizing his/her review and any recommendations. This report was provided to the study sponsor and the responsible IRBs. After review of the report for the first 5 participants enrolled, the sponsor permitted the study to resume enrollment.

Following this initial review, and at the request of the local IRB, two local physicians with relevant expertise will be appointed as independent safety monitors (ISMs) to assist in the continuing review of bronchoscopy safety data for study participants. The ISMs will receive all protocol revisions and may receive other documents related to the study, as necessary. The ISMs will not be directly involved with the study, not under the investigator's supervision, preferably in a different department and will have no financial, intellectual, proprietary or professional interest in outcome of the study.

The ISMs will review for safety after every 10 participants who have undergone bronchoscopy. Participants' safety records such as the pre-bronchoscopy, bronchoscopy, and follow up notes, as well as the laboratory test notes and any other adverse experience occurring during and after bronchoscopy will be reviewed. The two ISMs will each prepare a report following their review of safety data to document their individual recommendations regarding continuation of the study. The study coordinator, in consultation with the investigator, will provide the relevant participant data for review in a format that ensures confidentiality of the study data, and will facilitate the preparation and distribution of the recommendation reports. The written reports will be provided to the PI(s), investigators, and IRBs.

If the two local ISMs fail to reach consensus in favor of a recommendation concerning continuation of the study, another experienced physician based at the University Hospital Case Medical Center (UHCMC) who is not a study investigator, will be contacted to review the study information and to provide an overall recommendation regarding study continuation. This third opinion will serve as the overall recommendation, and also be provided to the PI(s), investigators, and IRB(s), and will serve as the final recommendation.

Enrollment will not be halted unless any of the study stopping rules listed in Section 9.4 are met.

10.0 CLINICAL MONITORING

10.1 Site Monitoring

Site monitoring will be conducted by the sponsor's designee and/or Coordinating Center personnel as applicable to ensure that the protection of human participants, study procedures, laboratory procedures, study intervention administration, data collection, and documentation are of the highest standard and conducted in accordance with the study protocol, SOPs, NIAID/DMID policies/procedures, ICH/GCP guidelines and applicable Ugandan regulatory guidelines.

Site monitors will have access to the study site, study staff, and all study documentation as per site monitoring plan. The timing and intervals of monitoring visits will be determined by the Coordinating Center and/or the sponsor's designee as appropriate. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, case report forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions. The investigators will undertake corrective action to resolve any problems identified by the monitor in consultation with the Investigators, Coordinating Center and the sponsor.

11.0 STATISTICAL CONSIDERATIONS

11.1 Study Outcome Measures

Outcomes:

Immunological Parameters:

1. Cytokine protein and mRNA levels in peripheral blood from LTBI, CNVT, and RLTBI.
2. Cytokine protein and mRNA levels in BAL fluid from LTBI, CNVT, and RLTBI.
3. Phenotypic analysis of cells in BAL fluid and peripheral blood samples from LTBI, CNVT, and RLTBI.
4. T cell responses to MTB antigens in BAL fluid and serum by ELISPOT and ELISA from LTBI, CNVT, and RLTBI.

Microbiological Parameters:

1. Presence of viable MTB by culture of BAL fluid on solid and liquid culture media from LTBI, CNVT, and RLTBI.
2. Presence of MTB proteins, DNA and (glyco-) lipids BAL fluid from LTBI, CNVT, and RLTBI.

11.2 Sample Size Considerations

Twenty-five participants in each cohort of LTBI, CNVT and RLTBI, will be enrolled into the study. Comparison of IFN-gamma levels between infected (LTBI and/or CNVT) and RLTBI will be an unmatched comparison of means (t-test). The power is based on a 2-sided test with $\alpha=0.05$. The null hypothesis is no difference between group means. IFN-gamma means and standard deviations in the power calculation were based on a previous study by Mahan et al. [28]. The power to detect a difference in BAL fluid IFN-gamma levels between infected (LTBI and/or CNVT) and RLTBI is shown in figure 3. 80% power can be achieved given a 1 log difference in mean pg/ml cytokine and a standard deviation of 1.0 or a 0.5 log difference with a standard deviation of 1.5 with a fixed sample size of 20 per group.

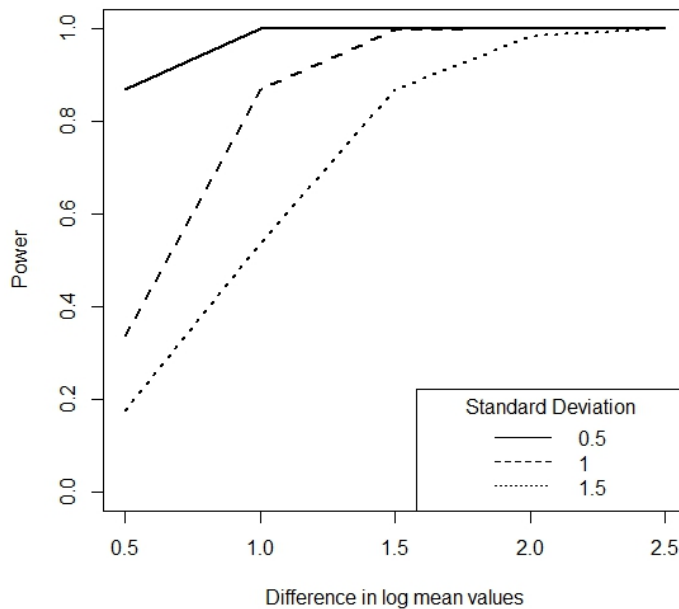


Figure 3: The power to detect a true difference in log mean IFNgamma responses between TST positive household contacts and contacts with at least 12 months of negative TST readings is shown as a function of the absolute difference in log mean values and the standard deviation with a sample size of 20 participants per group. A range of estimates for the mean and standard deviation were generated using IFNgamma responses to culture filtrate, antigen 8b5 and CFP10 presented in Mahan, et al, 2012.

11.3 Participant Enrollment and Follow-Up

Participants will be selected as described in Section 5.0. The target enrollment is 25 LTBI, 25 CNVT, and 25 RLTI. One follow-up visit will occur related to the safety of the bronchoscopy procedure, within 72 hours following the procedure. There will be no longer-term follow-up for this study.

11.4 Analysis Plan

Initial descriptive data analysis will be done to investigate distributions of the immunologic and microbiologic data. Non-normal data will be log-transformed and/or evaluated using non-parametric tests. Statistical analyses will be done using SAS (SAS Institute, Cary NC), SPSS for Windows (SPSS Inc, Chicago, IL) and R (R Foundation of Statistical Computing, Vienna, Austria) statistical packages. Tools for gene ontology, pathway, network and integrative exploratory analysis will be used as appropriate.

Group comparisons of cytokines, T-cell responses to MTB antigens, and specific mRNA transcripts between infected (LTBI and/or CNVT) vs RLTI groups will be done using t-test for independent means or the Wilcoxon-Mann-Whitney test if the data are not normally distributed. Within participant comparisons of whole blood cytokine results (IFN- γ , TNF- α , IL-6, and chemokines) with BAL fluid cytokine results will be done using paired comparisons of means or medians. Two-way analysis comparing LTBI/RLTI, CNVT/RLTI, and BAL/whole blood groups simultaneously will utilize a random effects ANOVA model. The proportion of culture positive individuals based on bronchoscopy will be estimated within LTBI, CNVT, and RLTI groups. The chi-square test will be used to test for an association between culture positivity and infection status.

Other measures including cell phenotypes, abundance of MTB molecules and exploratory transcriptomics and metabolomics will be analyzed using descriptive and graphical techniques without formal statistical tests.

12.0 ACCESS TO SOURCE DATA/DOCUMENTS

ICH/GCP 1.51 defines source documents. Source documents include original documents, data, and records such as clinical charts, laboratory notes, recorded data from automated instruments, x-rays, and participant files. The sponsor and/or sponsor's authorized representatives, Coordinating Center staff, and applicable regulatory agencies may have access to clinical records for the purpose of monitoring, auditing, quality assurance reviews and overall evaluation of the study safety and

progress. During monitoring visits, the monitor will have access to all source documents (data collection forms) held at the study site.

13.0 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Quality Control and Assurance Management

The investigative sites UCRC and the TBRU CWRU are responsible for conducting routine quality control (QC), and quality assurance (QA) activities, as described in the TBRU CWRU QMP, to internally monitor study progress, protocol, and ICH E6 Good Clinical Practice compliance. The TBRU CWRU QMP is maintained at the TBRU Coordinating Center at CWRU.

The Principal Investigator 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. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Data will be collected and maintained as described in the Coordinating Center's Data Management Plan (DMP) for this protocol.

14.0 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

14.1 Ethical Standard

This study will be conducted in compliance with the Declaration of Helsinki and the Ugandan National Guidelines for Research Involving Human Subjects, published by the Ugandan National Council of Science and Technology.

The investigator(s) will ensure that this study is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 312, and/or ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board (IRB)

The protocol and informed consent documents (Appendix B) and any subsequent modifications will be reviewed and approved by the duly-constituted Institutional Review Boards or Ethics Committees with active U.S. Federal Wide Assurances (FWAs) at the Ugandan study site in Kampala, Uganda and at the UHCMC IRB in Cleveland, Ohio. The protocol and any revisions will also be reviewed by the Uganda National Council for Science and Technology (UNCST) according to their policies and procedures.

14.3 Informed Consent

Each study participant must give written informed consent for participation in the study. The informed consent will be written in lay terms. The informed consent will be obtained prior to enrollment into the study. The informed consent form will be read by a study team member to clients who cannot read, with a witness to the consent of the illiterate participant. In that case, documentation of informed consent will be consistent with 45CFR117 (b). Informed consent documents will be translated verbatim into Luganda, one of the most common local languages in the area, for participants who are not able to speak and/or read English, and back-translated to assure accuracy in translation. The participant will have an opportunity to ask questions and raise any concerns about the study with study personnel. The informed consent form will be signed by both the participant and investigator and by a witness if the participant is illiterate. The witness will not be a study team member, but may be a family member or site staff member who is not part of the study team. The illiterate participant will be asked to mark the consent document with a thumbprint, and the witness will sign attesting that the

thumbprint was affixed by the participant. A copy of the informed consent form will be given to each participant.

The consent will include information about fiber optic bronchoscopy and detailed information regarding the risks and benefits of bronchoscopy and the bronchoscopy procedure itself.

Informed consent documents to be used in this study, including consent for study participation and consent for future use specimens, are included in Appendix B.

Appropriate pre- and post-HIV test counseling of all study participants will be performed by trained study personnel who are clinical staff trained as nurses that have extensive experience in HIV testing.

14.4 Exclusion of Children

Participants 18-50 years old will be eligible for enrollment. Children will not be enrolled to this protocol. The risk of research bronchoscopy would pose an unwarranted health risk out of proportion to the information to be gained for participants under the age of 18.

14.5 Participant Confidentiality

Participant confidentiality will be maintained. Research records, blood, and BAL samples will contain only participant ID numbers and participant initials. Original data entry forms and participant consents will be stored and maintained in locked cabinets or locked areas of the Ugandan project office, by the study team in Uganda. Participant names will not be used on CRFs and will not be entered into study research databases to protect participant identity. Databases containing data collected during the study will be maintained on secure servers that are password protected. The key that links the participant number to the participant identity will be maintained in a separate locked cabinet in the Ugandan project office.

Study-related information will be kept confidential except in a few circumstances. Information concerning the participant, the participant's identity, medical history and information collected during this study may be disclosed if required by law. Such information may also be disclosed or used by others involved in or overseeing the study, the study sponsor and its agents, as well as U.S., governmental, regulatory and accrediting agencies. Records may be reviewed by the following groups:

- U.S. Office for Human Research Protections, National Institutes of Health and/or its designee, or other U.S. federal, state or international regulatory agencies
- Uganda Makerere University School of Biomedical Sciences Higher Degrees Research and Ethics Committee
- Ugandan National Council for Science and Technology
- UHCMC IRB
- Case Western Reserve University

14.6 Cost/Participant Compensation/Research Related Injuries

Participants will be compensated monetarily for their participation in this study. A participant may be provided transportation to the clinic or compensated for transportation costs up to 20,000 Ugandan shillings on study visit days.

Participants who attend and undergo the bronchoscopy will be compensated 55,500 Ugandan shillings. Compensation for the bronchoscopy study visit is higher because of the extended length of the visit and the four-hour observation period that follows the procedure. This compensation will be reviewed at the time of each study renewal to be certain the amount offered is still appropriate.

There will be no cost to participants for examinations, laboratory testing and clinical care if indicated while on-study. Any costs for hospitalization or treatment for medical problems that participants may have that are not related to the study will be their own responsibility.

If a participant is injured as a result of participation in this study, the study clinic will give them immediate necessary treatment for their injuries, including coverage of cost of treatment for any related injury.

14.7 Future Use of Stored Specimens

As a part of the informed consent process, participants will be given the option to allow or not allow future use of stored samples. Per Ugandan requirements, a separate consent form for retention/storage of samples for future TB research will be used for this purpose.

Participants will be given a choice during the informed consent process to have their residual linked samples stored indefinitely for future use or have their residual linked samples delinked (anonymized) and stored indefinitely for future use or have their samples destroyed at completion of the study. If a participant does not consent to future use of stored samples, the participant may still participate in the study. If future research studies are considered for these residual long-term stored specimens, the IRB will review any additional research before it is conducted.

15.0 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

Data will be collected and maintained as described in the Coordinating Center's Data Management Plan (DMP) for this protocol, the QMP and in compliance with the International Conference on Harmonisation (ICH), Good Clinical Practice, Section 4.9. Study and data management staff will be trained to comply. Further information on data management for this study is found in more detail in the study DMP.

15.2 Data Capture Methods

Data from source documents will be entered onto standardized CRFs. Source documents may include laboratory reports, hospital records, and clinical memoranda. Data reported in the CRF derived from source documents should be consistent with the source documents or the discrepancies will be explained. OpenClinica™, a clinical data management system, will be used for data entry. OpenClinica incorporates double data entry and data cleaning rules for quality control as well as an automated audit trail. One-hundred percent of fields are reviewed during data entry. For CRFs that are not source documents, data will be abstracted from the clinical chart, clinical log, or laboratory logs. Where source documents are not utilized, entries will be made directly in the CRF, which will be regarded as the source document for the purposes of the study. The CRF will be the primary record of the participant's participation in the study.

15.3 Types of Data

Clinical and laboratory data will be collected for the study procedures and to document screening and enrollment procedures described herein.

Examples of Types of Data Collected

Type of Data	Purpose and Description
Adverse Event and Serious Adverse Event	Document adverse events and serious adverse events Severity of adverse events Relationship to study procedures
Chest X-ray	Document extent of chest x-ray and lung zones involved
Clinical Assessment	Provides examination of participant, clinical signs and symptoms and medical history
Demographics	Document information on age and sex
Endpoint	Document clinical endpoints and outcomes
Enrollment	Document eligibility criteria and enrollment date and enrollment processes

Laboratory Results	Document result of laboratory data such as HIV testing and complete blood counts
Protocol Deviation	Document each deviation that occurs for a participant to identify areas for further in-servicing and for reporting purposes
Risk Factors	Assess risk factors for bronchoscopy procedure
Vital Signs	Document vital signs such as weight and blood pressure
Withdraw	Includes information on early discontinuation of study follow up

15.4 Timing/Reports

The Independent Safety Monitors (ISMs) will review the bronchoscopy safety data after each 10 participants that have undergone bronchoscopy and BAL and report to the investigators, sponsor and IRB. A safety report will be provided to the investigators and will also be provided to the sponsor and IRBs as appropriate.

15.5 Study Records Retention

All study CRFs and documentation related to the study will be retained by the Uganda project site and Coordinating Center and archived in a secure storage facility for three (3) years following the completion date of the study, unless otherwise instructed by the sponsor.

15.6 Protocol Deviations

A protocol deviation will be defined as any action or inaction that is not in compliance with the protocol-specific requirements or Good Clinical Practice (GCP), the noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site PI/study staff to use continuous vigilance to identify and report deviations promptly to the Coordinating Center. The Coordinating Center will prepare reports of unanticipated problems and major deviations for the local IRB(s) per their reporting requirements. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. The Coordinating Center will provide protocol deviation reports to the sponsor and/or their designees as requested.

Exemptions for inclusion and exclusion criteria stipulated in the protocol will not be allowed. Protocol deviations will be detected by ongoing vigilance of the study staff, review of participant CRFs prior to data capture, review of adverse events by investigators, and internal QC procedures. The investigators will undertake prompt action to correct any identifiable factors underlying protocol deviations, which may include re-training of study staff where deemed necessary. All protocol deviations as defined above, possible sequelae, and corrective actions, will be recorded in the participant's source documentation. All protocol deviations will be reviewed by the PI or designated investigator to determine whether the data from that participant should be included in the analysis of the study endpoints.

All deviations that meet IRB/EC reporting requirements will also be sent to the sponsor Project Manager and Medical Officer.

16.0 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later

than 12 months after publication. Participant identifiers will not be included in any publications.

This policy applies to any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity, would be exempt from this policy.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Stage I trials), would be exempt from this policy.

If required by the sponsor for this study, the PI may be required to register the trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA). University policy and practices require publication and dissemination of the results of the research.

17.0 References

1. Global Tuberculosis Report 2018, Geneva: World Health Organization. 2018. Licence: CC BY-NC-SA 3.0 IGO.
2. Ma, N., et al., Clinical and epidemiological characteristics of individuals resistant to M. tuberculosis infection in a longitudinal TB household contact study in Kampala, Uganda. *BMC Infect Dis*, 2014. 14: p. 352.
3. Kamat, S.R., et al., A controlled study of the influence of segregation of tuberculous patients for one year on the attack rate of tuberculosis in a 5-year period in close family contacts in South India. *Bull World Health Organ*, 1966. 34(4): p. 517-32.
4. Tameris, M.D., et al., Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*, 2013. 381(9871): p. 1021-8.
5. Al-Arif, L.I., et al., HLA-Bw15 and tuberculosis in a North American black population. *Am Rev Respir Dis*, 1979. 120(6): p. 1275-8.
6. Fuller, C.L., J.L. Flynn, and T.A. Reinhart, In situ study of abundant expression of proinflammatory chemokines and cytokines in pulmonary granulomas that develop in cynomolgus macaques experimentally infected with *Mycobacterium tuberculosis*. *Infect Immun*, 2003. 71(12): p. 7023-34.
7. Hirsch, C.S., et al., In vitro restoration of T cell responses in tuberculosis and augmentation of monocyte effector function against *Mycobacterium tuberculosis* by natural inhibitors of transforming growth factor beta. *Proc Natl Acad Sci U S A*, 1997. 94(8): p. 3926-31.
8. Schwander, S. and K. Dheda, Human lung immunity against *Mycobacterium tuberculosis*: insights into pathogenesis and protection. *Am J Respir Crit Care Med*, 2011. 183(6): p. 696-707.
9. Drew, W.L., et al., Diagnosis of *Pneumocystis carinii* pneumonia by bronchopulmonary lavage. *JAMA*, 1974. 230(5): p. 713-5.
10. Finley, T.N. and A.J. Ladman, Low yield of pulmonary surfactant in cigarette smokers. *N Engl J Med*, 1972. 286(5): p. 223-7.
11. Finley, T.N., et al., Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. *Ann Intern Med*, 1967. 66(4): p. 651-8.
12. Golde, D.W., et al., Occult pulmonary haemorrhage in leukaemia. *Br Med J*, 1975. 2(5964): p. 166-8.
13. Golde, D.W., T.N. Finley, and M.J. Cline, The pulmonary macrophage in acute leukemia. *N Engl J Med*, 1974. 290(16): p. 875-8.
14. Huber, G.L., L.H. Edmunds, Jr., and T.N. Finley, Effect of experimental saline lavage on pulmonary mechanics and morphology. *Am Rev Respir Dis*, 1971. 104(3): p. 337-47.
15. Reynolds, H.Y., Use of bronchoalveolar lavage in humans--past necessity and future imperative. *Lung*, 2000. 178(5): p. 271-93.
16. Reynolds, H.Y. and H.H. Newball, Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med*, 1974. 84(4): p. 559-73.
17. Djukanovic, R., et al., The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am Rev Respir Dis*, 1991. 143(4 Pt 1): p. 772-7.
18. Elston, W.J., et al., Safety of research bronchoscopy, biopsy and bronchoalveolar lavage in asthma. *Eur Respir J*, 2004. 24(3): p. 375-7.
19. Hattotuwa, K., et al., Safety of bronchoscopy, biopsy, and BAL in research patients with COPD. *Chest*, 2002. 122(6): p. 1909-12.
20. Moore, W.C., et al., Safety of investigative bronchoscopy in the Severe Asthma Research Program. *J Allergy Clin Immunol*, 2011. 128(2): p. 328-336 e3.
21. Hunninghake, G.W. and R.G. Crystal, Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med*, 1981. 305(8): p. 429-34.
22. Reynolds, H.Y., Bronchoalveolar lavage and other methods to define the human respiratory tract milieu in health and disease. *Lung*, 2011. 189(2): p. 87-99.

23. Stover, D.E., et al., Diagnosis of pulmonary disease in acquired immune deficiency syndrome (AIDS). Role of bronchoscopy and bronchoalveolar lavage. *Am Rev Respir Dis*, 1984. 130(4): p. 659-62.
24. Stover, D.E., et al., Bronchoalveolar lavage in the diagnosis of diffuse pulmonary infiltrates in the immunosuppressed host. *Ann Intern Med*, 1984. 101(1): p. 1-7.
25. Wainwright, C.E., et al., Safety of bronchoalveolar lavage in young children with cystic fibrosis. *Pediatr Pulmonol*, 2008. 43(10): p. 965-72.
26. Hirsch, C., Ribeiro-Rodrigues, R, ResendeCo T, Valle Dettoni V, Haddad D, Johnson JL, Palaci M, Dietze R, Boom WH, Toossi Z, IFN-gamma, TNF-alpha and TGF-beta in the Tuberculosis Lung: Sputum Reflects Bronchoalveolar Lavage Cytokines. Unpublished.
27. Lewinsohn, D.A., et al., Whole blood interferon-gamma responses to mycobacterium tuberculosis antigens in young household contacts of persons with tuberculosis in Uganda. *PLoS One*, 2008. 3(10): p. e3407.
28. Mahan, C.S., et al., Innate and adaptive immune responses during acute M. tuberculosis infection in adult household contacts in Kampala, Uganda. *Am J Trop Med Hyg*, 2012. 86(4): p. 690-7.
29. Stein, C.M., et al., Linkage and association analysis of candidate genes for TB and TNFalpha cytokine expression: evidence for association with IFNGR1, IL-10, and TNF receptor 1 genes. *Hum Genet*, 2007. 121(6): p. 663-73.
30. Whalen, C.C., et al., Immune correlates of acute Mycobacterium tuberculosis infection in household contacts in Kampala, Uganda. *Am J Trop Med Hyg*, 2006. 75(1): p. 55-61.
31. Rose, A.S. and K.S. Knox, Bronchoalveolar lavage as a research tool. *Semin Respir Crit Care Med*, 2007. 28(5): p. 561-73.
32. Baughman, R.P., Technical aspects of bronchoalveolar lavage: recommendations for a standard procedure. *Semin Respir Crit Care Med*, 2007. 28(5): p. 475-85.
33. Hinckley, R., Case Report of Death of University of Rochester Student. 1996, NY State Department of Health.
34. Schwander, S.K., et al., T lymphocytic and immature macrophage alveolitis in active pulmonary tuberculosis. *J Infect Dis*, 1996. 173(5): p. 1267-72.
35. Schwander, S.K., et al., Enhanced responses to Mycobacterium tuberculosis antigens by human alveolar lymphocytes during active pulmonary tuberculosis. *J Infect Dis*, 1998. 178(5): p. 1434-45.
36. Society, B.T., British Thoracic Society Guidelines on Diagnostic Flexible Bronchoscopy. *Thorax*, 2001. 56 suppl 1: p. i1-i2.
37. Credle, W.F., Jr., J.F. Smiddy, and R.C. Elliott, Complications of fiberoptic bronchoscopy. *Am Rev Respir Dis*, 1974. 109(1): p. 67-72.
38. Lukomsky, G.I., A.A. Ovchinnikov, and A. Bilal, Complications of bronchoscopy: comparison of rigid bronchoscopy under general anesthesia and flexible fiberoptic bronchoscopy under topical anesthesia. *Chest*, 1981. 79(3): p. 316-21.
39. Pue, C.A. and E.R. Pacht, Complications of fiberoptic bronchoscopy at a university hospital. *Chest*, 1995. 107(2): p. 430-2.
40. Simpson, F.G., et al., Postal survey of bronchoscopic practice by physicians in the United Kingdom. *Thorax*, 1986. 41(4): p. 311-7.
41. Suratt, P.M., J.F. Smiddy, and B. Gruber, Deaths and complications associated with fiberoptic bronchoscopy. *Chest*, 1976. 69(6): p. 747-51.
42. Facciolo, N., et al., Incidence of complications in bronchoscopy. Multicentre prospective study of 20,986 bronchoscopies. *Monaldi Arch Chest Dis*, 2009. 71(1): p. 8-14.
43. Kim, C.K., Y.Y. Koh, and Z. Callaway, The validity of induced sputum and bronchoalveolar lavage in childhood asthma. *J Asthma*, 2009. 46(2): p. 105-12.
44. St-Laurent, J., et al., Alveolar macrophage subpopulations in bronchoalveolar lavage and induced sputum of asthmatic and control subjects. *J Asthma*, 2009. 46(1): p. 1-8.
45. Lensmar, C., et al., Leukocyte counts and macrophage phenotypes in induced sputum and bronchoalveolar lavage fluid from normal subjects. *Eur Respir J*, 1998. 12(3): p. 595-600.

46. Du Rand, I.A., et al., Summary of the British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults. *Thorax*, 2013. 68(8): p. 786-7.
47. Awotedu, A.A., et al., Flexible fibre optic bronchoscopy in Ibadan: report of 6 years' experience. *Afr J Med Med Sci*, 1989. 18(2): p. 155-8.
48. Carr, I.M., et al., Blood loss during flexible bronchoscopy: a prospective observational study. *Respiration*, 2012. 84(4): p. 312-8.
49. Daley, C.L., et al., Pulmonary complications of HIV infection in Dar es Salaam, Tanzania. Role of bronchoscopy and bronchoalveolar lavage. *Am J Respir Crit Care Med*, 1996. 154(1): p. 105-10.
50. Kibiki, G.S., et al., Aetiology and presentation of HIV/AIDS-associated pulmonary infections in patients presenting for bronchoscopy at a referral hospital in northern Tanzania. *East Afr Med J*, 2007. 84(9): p. 420-8.
51. Reeve, P.A., Lung cancer in Malawi: experience of bronchoscopy in Zomba General Hospital. *Cent Afr J Med*, 1989. 35(3): p. 355-8.
52. Teklu, B., Flexible fibreoptic bronchoscopy in Addis Ababa: a 4-year experience. *Br J Dis Chest*, 1986. 80(3): p. 283-7.
53. Worodria, W., et al., Bronchoscopy is useful for diagnosing smear-negative tuberculosis in HIV-infected patients. *Eur Respir J*, 2010. 36(2): p. 446-8.
54. Cattamanchi, A., et al., Bronchoalveolar lavage enzyme-linked immunospot for diagnosis of smear-negative tuberculosis in HIV-infected patients. *PLoS One*, 2012. 7(6): p. e39838.
55. Taylor, S.M., et al., Low prevalence of *Pneumocystis pneumonia* (PCP) but high prevalence of *pneumocystis dihydropteroate synthase* (dhps) gene mutations in HIV-infected persons in Uganda. *PLoS One*, 2012. 7(11): p. e49991.
56. Taylor, S.M., et al., Low prevalence of *Pneumocystis jirovecii* lung colonization in Ugandan HIV-infected patients hospitalized with non-*Pneumocystis pneumonia*. *Diagn Microbiol Infect Dis*, 2012. 72(2): p. 139-43.
57. Deok-jong Yoo, S., et al., The prevalence and clinical course of HIV-associated pulmonary cryptococcosis in Uganda. *J Acquir Immune Defic Syndr*, 2010. 54(3): p. 269-74.
58. Kyeyune, R., et al., Causes of early mortality in HIV-infected TB suspects in an East African referral hospital. *J Acquir Immune Defic Syndr*, 2010. 55(4): p. 446-50.
59. Worodria, W., et al., The role of speciation in positive Lowenstein-Jensen culture isolates from a high tuberculosis burden country. *PLoS One*, 2011. 6(11): p. e27017.
60. Mtunthama, N., et al., Malawians permit research bronchoscopy due to perceived need for healthcare. *J Med Ethics*, 2008. 34(4): p. 303-7.
61. CDC, Global Tuberculosis Report 2015, WHO, Editor. 2015.

APPENDIX A. TIME AND EVENTS SCHEDULE

	Screening Visit Day 0	Baseline Eligibility Assessment ¹ +7-14 days	Pre- bronchoscopy Visit +1-14 days	Bronchoscopy Visit +1-3 days	Post- bronchoscopy Visit +1-3 days
Counseling & Consent ²	X				
Demographic Information, Medical History, Targeted Physical Exam, Vital Signs, SpO2	X		X	X	X
Chest Radiograph ³	X				
Rapid HIV Testing	X				
HIV confirmatory Testing (if HIV+)	X				
CBC/Diff	X		X		
ALT/total bilirubin/serum creatinine	X				
PT/aPTT	X		X		
IGRA Testing	X ⁴			X ⁵	
Urinary pregnancy test for women	X		X		
Sample Storage (blood)				X	
BAL fluid collection/storage				X	

1. The baseline assessment time point will determine eligible participants for bronchoscopy. No visit is necessary; participants will be contacted by telephone to confirm eligibility and willingness to undergo bronchoscopy as well as scheduling of the next visit.
2. Counseling and consent will occur during screening. Confirmation of consent will occur during subsequent visits before proceeding with the bronchoscopy procedure.
3. If past chest radiograph available within preceding 6 months from ongoing household contact study, result will be abstracted. If no chest radiograph available, chest x-ray will be performed.
4. Abstraction of IGRA result at screening from ongoing household contact studies.
5. Blood collected at bronchoscopy study visit will be utilized for immunologic testing e.g. IGRA. IGRA performed for all cohorts CNVT, RLTBI, and LTBI.

APPENDIX B. CONSENTS

General Consent

INVESTIGATORS

Harriet Mayanja-Kizza, MBChB, MMed, PhD
Makerere University, Kampala, Uganda
Telephone: 256-414-534-262

W. Henry Boom, MD
Case Western Reserve University
Cleveland, Ohio 44106
Telephone: +1-216-368-1949

I. PURPOSE

This form gives you information about a research study that will also be discussed with you.

The doctors are studying people who live with someone who is sick with tuberculosis (TB). TB is a serious infection caused by the TB germ. It can cause serious disease in the lungs and other important parts of the body. TB disease is very common in Uganda and affects men, women, and children. Persons with TB disease often have a very bad cough. TB disease is spread from one person to another when a person breathes air containing TB germs coughed out by someone with TB disease. Often, people who live with someone who has TB disease get infected with the TB germ themselves, but sometimes they do not.

You are being asked to join this study because you have already joined another study looking at people who live with someone who is sick with TB. This research study is studying TB and how it spreads among people. The research doctors will do that by studying how your lungs may fight the TB germ, using a procedure called “bronchoscopy.” More information about the bronchoscopy is given below.

This consent form gives you information about the study, the ways the doctors will check your health, and how they will study your lungs with a procedure called bronchoscopy. This information will also be discussed with you. Once you understand the study, and if you agree to join, you will be asked to sign this consent form. You will be given a copy of the form to keep. Being in the study is voluntary and will not affect your medical care or joining any other research studies in the future. You may also leave the study any time you want.

This study is being done by doctors from Uganda and the United States. About 75 people are expected to join this study. Your time on this study will last no more than 3 weeks from the time you agree to join the study through the last visit to check your health.

II. STUDY PROCEDURES

Screening Visit:

The doctors will do a physical examination to study your health. This examination will allow the doctors to see if you are sick with TB or not. The details of what will be done at this examination are talked about below.

The doctors will also examine you later in the study to check on your health, before and after you have the study procedure called bronchoscopy.

At your screening (first) visit, the following health checks will be done. You will already have had a TB skin test as part of the other study you joined, and the skin test will not be repeated as part of this study.

- The doctors will examine you and ask you questions about your health and any other illnesses you have. They will ask if you take any medication.
- Measure your height, weight and give you a general check-up.
- Do a blood test to check if you are infected with HIV, the germ that causes AIDS. You must have an HIV test to join this study. You will be tested for HIV even if you have been tested somewhere else before this. Before the test is done, a counselor will talk with you about why we need to do the test and what the results mean. The counselor will give you the result of the test, and the clinic staff working on this study will learn your result also. You may ask the doctor or counselor questions about the HIV test at any time. The results of the HIV test will never have your name on them.
- If you do have HIV, you will not be able to join the study. If you have HIV, you can still be treated for your HIV and TB (if you have it) through the Uganda National Tuberculosis and Leprosy Programme (NTLP). You will be told where you can go for treatment and medication, if needed. People infected with HIV usually go to Immune Suppression Syndrome (ISS) Clinic for care, but you can choose another doctor or clinic for this care if you want.
- The nurses will take blood from your arm (about 4 to 5 mL or about 1 teaspoon). This blood will be used to check on your health.
- The doctors will take some of your urine to see if you are pregnant if you are a woman. If you are pregnant, you will not be able to join the study.
- The doctors will take an X-ray of your lungs to see if you have TB disease in your lungs. If you have had a chest X-ray done within the previous 6 months, the physician will not ask you to have another one unless they think you may have gotten sick with TB recently.

If you do agree to join the study, the doctors will contact you in one to two weeks to let you know if your health checks show that you are healthy enough to continue in the study. If your health checks show that you may have a condition or illness that prevents you from continuing on the study, the doctors will tell you where you can get care.

Pre-Bronchoscopy Visit:

If you continue on the study, the doctors will ask you to schedule a procedure called bronchoscopy. You will also be asked to come to the clinic between 1 and 3 days before the bronchoscopy to check on your health again. At this visit, the doctors will repeat the following:

- The doctors will examine you and ask you questions about any changes to your health and any other illnesses you have. They will ask if you take any new medications.
- You will be asked again if you want to continue with this study on this day.
- Measure your height, weight and give you a general check-up.
- The nurses will take blood from your arm (about 4 to 5 mL or about 1 teaspoon) to repeat health check testing.
- If you are a woman, a urine test will be repeated to see if you are pregnant.
- You will be given additional information sheets providing you with more details concerning the bronchoscopy procedure and procedure instructions.

Bronchoscopy Visit:

At the bronchoscopy visit, you will be asked again if you want to continue with this part of the study on this day. You can decide you don't want to have the bronchoscopy and you will be withdrawn from the study. There is no penalty to you if you decide not to have the bronchoscopy. You will have the bronchoscopy done and blood drawn (60 mL or 4 tablespoons) with some of it stored to study how your body fights the TB germ. Some of this blood will be used to see if you have been exposed to the TB germ. Some of your blood may be kept for other TB research in the future if you agree. You will be asked if you agree to this on a separate consent form.

Bronchoscopy is a procedure where a small tube with a light on the end of it is passed through your nose or mouth into your windpipe to look at your lungs and also take fluid samples for laboratory tests. During the bronchoscopy, samples of the fluid in your lungs will be collected. A part of your lungs will be washed with clean fluid and the fluid will be removed for tests. This is called bronchoalveolar lavage. These samples will be used to study how your body fights the TB germ. Bronchoscopy is a medical test that is done at large research hospitals nearly every day to diagnose patients with serious lung problems. The bronchoscopy is done by a doctor who specializes in lung diseases and who is specially trained to do the bronchoscopy. A nurse or other medical staff will help the doctor during the bronchoscopy.

You will not be allowed to eat or drink anything for at least six hours before the bronchoscopy so that your stomach will be empty at the time of this test. Your blood pressure, breathing, and heart beat will be checked before the bronchoscopy. The doctor will check on your health to be sure you are healthy enough to have the bronchoscopy done. An intravenous line will be placed in a vein in your arm before the bronchoscopy to allow the doctors to give you medications and fluids, if needed.

Before you do the bronchoscopy, your nose, throat, and airways will be numbed with a medication called lignocaine (also called lidocaine). Lignocaine is an anesthetic like the one dentists use to prevent pain when you have a tooth removed or one a doctor would inject around a cut before sewing up a wound. Lignocaine tastes bitter. Once your nostrils and throat are numb, the bronchoscope (the thin tube with a light on the end) is gently passed through one of your nostrils or mouth and into your windpipe. Small amounts of lignocaine will be sprayed through the bronchoscope to numb your windpipe and decrease coughing. You will not be able to speak while the bronchoscope is in your windpipe.

You will be given extra oxygen during the bronchoscopy by nasal cannula (two little plastic tubes inserted approximately a centimeter into your nose). The amount of oxygen in your body will be watched by the doctors and nurses throughout the bronchoscopy.

During the bronchoscopy, you may have some coughing and a feeling that it is hard to breathe. The doctors and nurses will watch you closely to be sure that you are breathing properly. Your heart beat and oxygen level will be measured during the entire bronchoscopy. The amount of oxygen in your blood will be measured with a clip lightly attached to your finger.

The lung washing will be done with small amounts of germ free (sterile) salt water that is put into a small part of the lung and then removed. The washing will be done a total of 8 times, and takes about 10-15 minutes. The samples of washing will be used for tests in the laboratory. The washings remove some loose cells that are found in your lungs. Your body quickly replaces the cells that are removed.

The bronchoscopy test takes about 50 minutes, including the time to numb the nose and throat before the bronchoscopy. After the bronchoscopy, you will be asked to stay at the clinic for 2 hours, or longer if the doctor requests, to be sure that you are doing well after the test. The doctors and nurses will watch you closely after the test to be sure that you are breathing properly. Your heart beat and oxygen level will be measured for several hours after the bronchoscopy. You will not be allowed to eat or drink for 3 hours after the test until the numbness in your throat is gone so that you won't choke.

Post Bronchoscopy Visit:

You will be asked to come to the clinic to meet with the nurses within 3 days after the bronchoscopy visit. At this visit, the nurses will check on your health. The nurse will check your blood pressure, heart rate and breathing, and will ask you questions about how you have been feeling. You will also have a brief physical exam to check your health.

III. STUDY OF YOUR BLOOD

As noted above, the doctors would like to take some of your blood to check on your health during the first two visits before the bronchoscopy is done and during the bronchoscopy visit. Some of this blood from the visit where you have the bronchoscopy will also be used to study how your body fights TB.

After the study is over, your samples will be stored and may be shared with other researchers. The samples will be available for any research question, such as research to understand what causes certain diseases (for example heart disease, cancer, or kidney disease), development of new scientific methods, or the study of where different groups of people may have come from.

You may give permission, or not, for your samples to be used in future research by other researchers. You can still join this study if you do not allow your samples to be used by other researchers in the future.

You will be offered a separate consent that tells you more about the possible future use of your samples.

IV. RISKS

Risks if you join this study:

1. The risk from drawing your blood may include discomfort from the needle stick, bleeding, bruising, dizziness, fainting (in less than 1 out of every 100 patients who get their blood drawn) or rarely infection (in less than 1 out of every 1,000 patients who get their blood drawn). If you prefer, the blood can be drawn at two different clinic visits.
2. You may have an x-ray of your chest to check on the health of your lungs, if one has not been done in the last 6 months, or if the doctors think you might have become sick with TB. These x-rays use a small amount of radiation. The amount of radiation you will receive in each of these x-rays is very small and hard to measure but still may be harmful if you have already had many x-rays in the past. If you have had many x-rays, you should discuss this with the doctors.
3. Testing for and learning your HIV status may cause you anxiety.
4. There is a risk that your identity could mistakenly be shown to people who do not work on this study. All care will be taken to prevent this from happening, including labeling your study records with a number instead of your name.

The following are risks related to the bronchoscopy procedure:

1. You may have some discomfort, coughing, chills, and shortness of breath during the bronchoscopy. You may have a sore throat or hoarse voice for a day or two after the procedure. Fever is common during the first night after the bronchoscopy, about 1 out of every 10 people who have a bronchoscopy get a fever afterwards. The fever usually lasts less than a day and can be treated with acetaminophen (also called paracetamol). Very rarely a patient may develop an infection following the bronchoscopy (less than 1 out of every 100 people who have a bronchoscopy). If

this happens, you will be treated for the infection. Significant bleeding occurs in less than 1 out of every 350 people who have a bronchoscopy. Other serious, but rare, problems that occur in less than 1 out of every 1,000 people who have a bronchoscopy include wheezing, vocal cord spasm, irregular heartbeat, collapse of a lung and significant bleeding. Death is rare, occurring in 1 out of every 5,000 people who have a bronchoscopy.

2. If you are allergic to the numbing medicine lignocaine, you could develop a rash, dizziness, lightheadedness, vomiting, and rarely, low blood pressure, a seizure, problems with your heart or difficulty breathing. Before the bronchoscopy, the doctors will ask you if you have had lignocaine before and if you had any problems from the medicine before. If you have a problem with the lignocaine during the bronchoscopy, the doctors will have medication available and will treat you. If the problem occurs before the bronchoscopy, the bronchoscopy will not be done.
3. If an irregular heartbeat, bleeding, collapse of the lung or other problems occur during the test, the study doctors and nurses will have medication and equipment ready to treat your problems. Your heart beat and oxygen levels will be checked during the test and for a minimum of 2 hours afterwards. If your oxygen level or your heartbeat requires treatment, you will be treated.

V. BENEFITS

You will have health checks that may help you learn if you have any serious health issues or if you have been infected with TB. There is no direct benefit to you by having the bronchoscopy done. However, the results of this study may help the doctors understand how the human body fights TB. This knowledge may be useful in finding better ways to prevent and treat TB.

VI. ALTERNATIVE PROCEDURES

You do not have to join this study. If you have been told you can't join this study because you have TB, HIV infection, or are pregnant, you will be told where you can go for additional care.

VII. RESEARCH RELATED INJURIES

You will be closely watched by the study doctors during the entire study. If you have any serious reactions to the bronchoscopy or the lung washing, you will be treated right away. If you have a serious medical problem due to the bronchoscopy or any procedure in the study, you may be admitted to the hospital for treatment.

You will be offered free medical treatment for any illness or injury that was caused by being in this study. This treatment will not cost you anything and will include any emergency treatment and any further care at a government hospital. This care will be given at the highest standard available in Uganda. If you choose to have further treatment at a private hospital or clinic, additional money that might be needed for these treatments will have to be paid by you.

VIII. COSTS AND COMPENSATION

You will not have to pay any money for the tests and health checks done as part of this study.

You will be given free transportation to and from the clinic for research visits. You will either be given a ride by the clinic staff, or you will be given 20,000 Ugandan shillings for each study visit to pay for the cost of transportation. If your study related costs exceed the specified amount and you have proof of such expenses, you will receive compensation equivalent to the out of pocket expenses.

If you agree to come to clinic for the bronchoscopy study visit, you will be provided 55,500 Ugandan shillings together with a food basket (rice, soya flour, sugar, cooking oil) to cover the cost of transport and for your time after the bronchoscopy during which you will be watched at the clinic.

IX. VOLUNTARY PARTICIPATION AND REASONS FOR WITHDRAWAL

Being in this study is voluntary. If you do not join this study you will not lose any treatments or other benefits that you would be entitled to otherwise. If you agree to join this study, you must try to keep all your appointments. If you decide to leave the study, you can leave at any time, for any reason without punishment or losing any benefits you would have if you did not join. The doctors might learn some new study results from studying other people in the study that could make you not want to be in the study anymore. If this happens, you will be told what the doctors found so that you can choose to continue or stop being in the study.

Also, the doctors might ask you to leave the study if they find you have a certain illness or take certain medications that could make it unsafe for you to stay on study. They will discuss with you any changes in your health or medications that may require you to leave the study.

X. CONFIDENTIALITY

Personal information will be collected from you, but only people working on the study will see it. All efforts will be made to keep the personal information in your research record private, but absolute confidentiality cannot be promised. If your records are reviewed your identity could become known, but people reviewing your records will not tell anyone your identity.

Research records will be kept confidential to the extent required by law. By joining this research study, you are giving permission to certain agencies and people to look at the research records for safety reasons. These agencies or people include the following: U.S. Office for Human Research Protections, National Institutes of Health and/or its designee, or other US. federal, state or international regulatory agencies, Uganda Makerere University School of Biomedical Sciences Higher Degrees Research and Ethics Committee, Ugandan National Council for Science and Technology, University Hospitals Cleveland Medical Center IRB, Case Western Reserve University and the professional

staff participating in the study.

XI. CONTACT PERSONS FOR QUESTIONS OR PROBLEMS

_____ has described to you what is going to be done, the risks, dangers and benefits involved. The doctor in charge of this study is Dr. Mayanja-Kizza. You may ask any questions you have now. If you have any questions, concerns or complaints about the study in the future, you should contact Dr. Mayanja-Kizza at the clinic or by phone at 256-414-534-262 if you have any concerns about your rights as a person joining a study.

The physicians performing the bronchoscopy procedure will be either Dr. Worodria or Dr. Opio. You may contact either one by phone at the clinic by calling 0750576655 or 0793 518 080.

If the doctors cannot be reached, or if you would like to talk to someone other than the study doctor(s) about questions about the study, your rights while being in the study, research-related injury, or any other questions, contact Dr Erisa Mwaka the School of Biomedical Sciences IRB chairman, by calling 075 257 5050 or the School of Biomedical Sciences Regulatory Office by calling 070 194 0363. You may also write Dr. Erisa Mwaka at PO Box 7072, Kampala, Uganda.

XII. SUMMARY OF YOUR RIGHTS AS A PARTICIPANT IN A RESEARCH STUDY

Joining this study is voluntary. Refusing to participate will not change your usual health care or cause any punishment or loss of benefits you would otherwise be entitled to. If you decide to join the study, you may leave the study at any time and for any reason without punishment or loss of benefits. If information from this study is published or otherwise shown to anyone, your identity will be kept secret. If the doctors find new information that may affect the risks or benefits of being in this study or your willingness to participate in it, you will be told so that you can decide whether or not to continue in the study.

XIII. AUTHORIZATION TO USE AND DISCLOSE YOUR INFORMATION

You authorize Dr. Mayanja-Kizza in Uganda, and other doctors working with her at University Hospitals Cleveland Medical Center (UHCMC), Case Western Reserve University and the people working for them to use and share information about you and your medical history and information collected during this study for the following purposes:

- to find out why some people get sick with TB and others don't get sick.

Such information may also be disclosed or used by others involved in or overseeing the study including the School of Biomedical Sciences Institutional Review Board, the UHCMC Institutional Review Board, the study sponsor and its agents, as well as U.S., your and other governmental, regulatory and accrediting agencies. Foreign laws governing privacy, use and disclosure of health information may provide less protection than the laws of your

country. Once disclosed your information may be re-disclosed by others who are not required to maintain the privacy of your information. You may withdraw authorization to collect additional information about you at any time by writing to the local Principal Investigator, but information already collected may continue to be used and disclosed. This authorization has no expiration date.

XIV. CONSENT

You have been given a copy of this form and know that a copy of this form will be kept in your study file. You may leave the study at any time without punishment. The study doctors may ask you to leave the study if they think it will be best for your health. They are asking you now to give permission to join the study.

Signing below says that you have been told about the research study and that you voluntarily agree to join; that you have asked any questions about the study that you may have; that you have been given all the information you need to make a decision about joining the study and that you have not been forced to join the study. When you sign this consent form, you do not give up any legal rights, and the investigator(s), doctors(s) or sponsor(s) are still responsible for anything bad that might happen to you as a result of this study. A copy of this consent form will be given to you.

(Subject's signature or mark should be placed on the line.)

I understand that by signing below or making my mark, I agree to join the study and follow the study procedures to the best of my ability.

_____ Signature of Participant / Thumb Print	_____ Date	_____ Printed Name of Participant
---	---------------	--------------------------------------

_____ Signature of Person Obtaining Consent	_____ Date	_____ Printed Name of Person Obtaining Consent
---	---------------	--

(Must be study investigator or individual who has been designated to obtain consent.)

_____ Signature of Witness (for illiterate subjects only)	_____ Date	_____ Printed Name of Witness
---	---------------	----------------------------------

CONSENT FOR RETENTION OF SAMPLES AND GENETICS RESEARCH

For Use with Bronchoscopy Study Consent

BACKGROUND

The doctors have explained that you may be able to join the bronchoscopy study. As part of that study, the doctors will obtain samples that may include blood and bronchoalveolar lavage (BAL) fluid, leftover from the lung washing that was described to you in the main study consent. After these tests are done, the doctors may have some amount of each sample left. They are asking to store what remains for future use.

SPECIFIC DETAILS REGARDING SAMPLES TO BE STORED

With your permission, the doctors would like to store any blood left over from your blood that is drawn at the bronchoscopy visit after this study is over. The blood draw amount is expected to be 60 ml (1 teaspoon to 4 tablespoons). The amount left over and stored will not be greater than 60 ml. The amount of blood left over may vary depending upon how many cells you have in your blood.

The doctors would also like to store lung fluid from your bronchoalveolar lavage (BAL) which they obtained during your bronchoscopy. The amount of BAL is not expected to be more than about 150-200 ml (10-13 tablespoons). They would use these specimens for studies they may do in the future to learn more about TB. These samples would be stored at the Joint Clinical Research Center in Kampala and may be shipped to Case Western Reserve University or other laboratories in the United States to test how your body fights the TB germ.

USE OF STORED SAMPLES FOR GENETIC STUDIES

In addition to using your blood and/or BAL to learn more about TB, the doctors may want to study some of these samples to study small parts of the body, called “genes.” In a family, people who share the same characteristics usually share the same genes in the blood.

- The scientific name for DNA is deoxyribonucleic acid. DNA provides unique information on how a human body functions. Specific parts of DNA are known as “genes”.
- Genes are in all people and are found in almost every part of the body, including the blood.
- These genes carry the information that passes characteristics from parents to their children. Genes carry information about characteristics that you see, like the color of your skin or your height; they also carry information about characteristics that you cannot see, like how well you can fight off the TB germ.

The researchers will not share any genetic information they learn about you with you or your family members. If you have family members who also join this study, the doctors may be able to see how closely you are related, but this information also will not be

given to you or your family members.

We may share portions of your DNA with other researchers working on different projects. If your DNA is shared with other researchers, your identity will not be given to the other investigators. Your DNA sample will be identified by a code number, and all other identifying information will be removed. The study doctors will keep a separate code sheet which links the DNA sample code number with your identity, but this will not be shared with the other researchers.

In addition to sharing information with other researchers, coded information about your DNA will also be submitted to a national database maintained in the United States at the National Institutes of Health (NIH) by the study researchers. This database is called GWAS. These coded samples will be shared with other investigators for future research purposes as part of this database. Neither NIH nor other researchers using the coded information will be able to identify you.

If, at a future date you wish to withdraw your consent to allow use of your DNA information in this NIH GWAS database, you can contact Dr. Mayanja-Kizza at the clinic by phone at 256-414-534-262 to withdraw your information. This will remove your information for any future research. Any research that was conducted with your data prior to this request will be unable to be withdrawn and may still be utilized.

It is unlikely at this stage that any future use of your DNA would give the researchers any information about you or your specific condition. Therefore, there are no plans for you to receive any individual results from any future tests. If a future researcher finds something very important about you or your condition, they may contact this study investigator with your code number and the results. The study investigator would then contact you regarding the results. Only your study investigator can connect the code to you.

WHAT THE RESEARCHERS ARE ASKING YOU

You can agree to allow the researchers to keep your blood and BAL to use in future TB research and for future genetic research. You can also say you will allow your samples to be kept and used only if all information about you, including your study number or other identifiers, is removed from the samples. They would not keep your study number, initials, age, sex or any data about your health problems. However, they would know that your samples came from a person from Uganda. The kinds of research the doctors can do with this kind of sample are more limited, but they still might be used. This means that you will not be able to change your mind in the future and tell the doctors to discard your specimens, since they will not be able to identify which samples belong to you.

Finally, you can say you don't want these samples saved and still join the Bronchoscopy study. If you choose not to allow the researchers to keep your blood and BAL for future TB and genetic research, you will still have blood and broncho-alveolar-lavage (BAL) samples taken as part of the Bronchoscopy study, as described in the study consent

form, but they will not be stored after the study is completed – the samples will be discarded.

You will be asked below whether you will allow the use of your samples for TB research and for genetic research separately. You can agree to allow your samples to be used only for TB research, only for genetic research, for both uses, or for neither.

OVERSIGHT OF SAMPLE STORAGE

If you agree to allow your samples to be stored for future TB and/or genetic research, your samples will not be given to anyone to study without permission by the School of Biomedical Sciences Institutional Review Board. If you do not agree to their storage, they will be discarded when the study is completed.

RISKS

If you agree to allow your samples to be stored, there is a small risk that your identity could become known. However, your samples will never have your name listed on them. Access to the freezer where they are stored, and the computer program that contains the information about your samples, is limited to trained laboratory technicians. These laboratory staff are not involved in the daily care of patients, and do not have access to your name or other medical information.

You may be anxious about having your genes tested to see what genes or family characteristics you share. In studying the genes, we may learn that some members of a family are not related.

The researchers will not tell you the results of any of the studies on your genes, or anything they learn about you or your family through your genes. They will not tell your household, your neighbors, other doctors, your family, or any other people the results. The results will not be put in the medical record, and they will not be used to diagnose you or treat any medical condition. The results will be used for research purposes only. Any information learned about your genes will be protected to the limits of the law in the United States and Uganda.

BENEFITS

There are no benefits to you for allowing your samples to be stored for future TB or genetic research. However, the information learned from studying your samples may help the Ministry of Health in Uganda, and health officials in other countries, to design better programs for TB control and treatment.

STATEMENT OF CONSENT:

I have been given a copy of this form and know that a copy of this form will be kept in my study file.

The researchers are asking you if they can keep some of your blood and BAL samples for future TB research and also for genetic research. The results of these tests will not

be used as part of your health care, and you will not be told the results. You can say that you do not want these samples saved, and can still be in the Bronchoscopy Study. Any future use of these samples would be reviewed by an Ethics Committee in Uganda to be sure their use is appropriate and protects your privacy.

Consent for Future Use in TB Research:

- ☐ I DO give permission for my blood and BAL samples to be stored with information about me included and be used in future TB studies.
- ☐ I DO give permission for blood and BAL samples to be stored and used in future TB studies only if all information about me that could be connected to the samples is destroyed first.
- ☐ I DO NOT give permission for my blood and BAL samples to be stored and used in future TB studies.

Consent for Future Use in Genetic Research:

Your DNA (genes) or your cells that can be used to make your DNA may be stored for future research purposes, either by the researchers or by other scientists working with the US National Institutes of Health. Please check one of the following options telling us how your DNA samples may be used.

- ☐ I DO give permission for my blood and BAL samples to be stored with information about me included and be used in future genetic studies.
- ☐ I DO give permission for blood and BAL samples to be stored and used in future genetic studies only if all information about me that could be connected to the samples is destroyed first.
- ☐ I DO NOT give permission for my blood and BAL samples to be stored and used in future genetic studies.

Signing below indicates that you have been informed about the research study in which you voluntarily agree to participate; that you have asked any questions about the study that you may have; and that the information given to you has permitted you to make a fully informed and free decision about your participation in the study. By signing this consent form, you do not waive any legal rights, and the investigator(s) or sponsor(s) are not relieved of any liability they may have. A copy of this consent form will be provided to you.

Signature of Participant / Thumb Print	Date	Printed Name of Participant
--	------	-----------------------------

Signature of Person Obtaining Consent	Date	Printed name of person Obtaining Consent
(Must be study investigator or individual who has been designated to obtain		

consent.)

Signature of Witness
(for illiterate subjects only)

Date

Printed Name of Witness