Bacille Calmette-Guerin (BCG) Vaccine for Immune Protection Against Infections

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JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
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1. Abstract

a. Provide no more than a one-page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Individuals with bronchiectasis, both due to cystic fibrosis (CF) and etiologies other than CF (non-CF bronchiectasis [NCFB]), are highly susceptible to recurrent respiratory infections which lead to morbidity and mortality. A concern for individuals with bronchiectasis are nontuberculous mycobacteria, which are environmental bacteria that can cause chronic lung infections that are difficult to eradicate.

The Bacille Calmette-Guerin (BCG) vaccine, given worldwide to infants and children to protect against tuberculosis (TB), has been shown to provide some protection against diseases other than TB by boosting the immune system, a phenomenon called "trained immunity" (TI). TI is the ability of a first infection to confer enhanced antigen-independent immunity in both myeloid and lymphoid cells. BCG is a potent stimulator of TI. Retrospective clinical trials have shown that BCG protects against HPV, RSV, influenza A, and HSV[1]. In addition, prospective trials have shown inhibition of replication by yellow fever virus vaccine strain[2].

Furthermore, in numerous human studies, BCG has been shown to confer cross-protection against nontuberculous mycobacteria (NTM) in healthy controls (HC). As NTM infection rates are increasing in cystic fibrosis (CF), and NTM infection leads to significant morbidity in CF, improved preventive approaches are needed. But as far as we know, there have been no studies that evaluate the use of the BCG vaccine against NTM in the CF and NCFB population. Our study explores whether BCG vaccination can elicit protective immune responses for NTM in CF and NCFB.

Our central hypothesis is that BCG vaccination will elicit in vitro markers of protective efficacy against NTM in CF and NCFB patients.

2. Objectives (include all primary and secondary objectives)

- **Primary objectives:**
  - To evaluate BCG uptake in adults with CF and NCFB as compared to healthy controls.

- **Exploratory objectives:**

- To evaluate whether BCG vaccination of CF and NCFB patients compared to healthy controls will show comparable immune responsiveness across multiple *in vitro* parameters of NTM protection and killing.
- To evaluate whether CF and NCFB patients demonstrate increases in trained immunity comparable to healthy controls following BCG vaccination.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Prevention of infection (POI) and disease (POD) by BCG against TB. The Bacille Calmette-Guérin (BCG) vaccine was developed in 1921 as a live attenuated vaccine for tuberculosis (TB) by French microbiologists Albert Calmette and Camille Guérin who serially passaged a virulent strain of *M. bovis*[3]. BCG is given intradermally to newborns and children in virtually all nations of the world except the US & Canada and certain countries in Western Europe. It is believed to be the single most widely utilized vaccine, with ~152 million doses given annually and an aggregate of at least 5 billion doses given[4]. Neonatal vaccination with BCG clearly confers protection from disseminated TB to children for ~5-7 years[5]. Importantly, BCG is making a major comeback for TB. For example, a recent NEJM study in which 990 high-risk adolescents free of latent TB infection (LTBI) received either BCG re-vaccination or a protein subunit+adjuvant known as H4:IC31 found that BCG revaccination conferred 45.4% POI, (p=0.03), which was far better than H4:IC31[6]. Similarly, intrabronchial BCG in a non-human primate model, demonstrated necropsy-proven POI (p=0.0018) while intradermal BCG failed in POI[7]. A Nature paper published in January 2020, showed that IV administration of BCG to rhesus macaques afforded complete POI against TB in the majority of animals[8]. These and other studies have prompted TB experts to dub BCG the "comeback kid" and consider repetitive BCG vaccination for further TB prevention studies[9].

BCG protects by enhancing "trained immunity." "Trained immunity" (TI) is the ability of a first infection to confer enhanced antigen-independent immunity in both myeloid and lymphoid cells[10]. TI changes include increased levels of (i) pro-inflammatory cytokine secretion, (ii) M1 polarization in MΦ,Φ, (iii) pro-inflammatory epigenetic changes, (iv) metabolomic shift towards anabolism in MΦ,Φ, and (v) autophagy. BCG is a potent stimulator of TI and has been shown to confer elevated responses in humans to heterologous challenges to agents such as yellow fever virus and tumor challenge and vaccines[2,7,11,12].

Rise of NTM in CF. For unidentified reasons, rates of pulmonary NTM are increasing, both in CF and NCFB patients[5,14,15]. Some of this increase may be due to improved clinician awareness, increased mycobacterial surveillance practices, and changes in environmental exposure or pathogenicity. However, another hypothesis for observed increases in NTM rates is that decreasing TB incidence and waning TB immunity may increase vulnerability to NTM infection.

Does BCG vaccination confer protection against NTM in humans? Recently, a prospective clinical trial in which 5 healthy people from St. Louis, MO were given intramuscular BCG observed post-vaccination increases in protective immunity against NTM[16]. Additional compelling human evidence for BCG-mediated protection against NTM comes from European nations with strong surveillance programs where BCG vaccination was suspended or terminated. In France, the mean incidence of culture-confirmed NTM cervical lymphadenitis sharply increased from 0.57 to 3.7 per 100,000 children per year after mandatory BCG vaccination was discontinued in 2007[17]**.** Similarly, in a large retrospective population-based study in Finland, when BCG policy changed from

universal to selective vaccination strategy in 2006, childhood NTM infections increased drastically, with an incident rate ratio of 19.03x (95% CI, 8.8-41.07; P<0.001)[18]. These studies support the hypothesis that BCG offers cross-protection against NTM disease in humans.

Lower NTM rate in CF in countries with standard BCG vaccination. We conducted an independent assessment of country-specific BCG vaccination policies per the WHO and NTM infection rates in 2019 as reported in the European CF Society Patient Registry[19,20]. Low rates of NTM infection are often observed in European countries that universally vaccinate for BCG, whereas higher rates are seen in countries that have ceased their standard BCG vaccination strategies, especially countries in which vaccination was suspended in the 1980s or 1990s. Similar low NTM rates are seen in CF patients in Brazil[21]. These findings support the hypothesis that BCG vaccination may protect against NTM infection in people with CF.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Study design: This is a prospective, single center, non-randomized open-label study.

Study population: We plan to recruit 60 participants with CF from the JH Adult Cystic Fibrosis Center, 60 participants with NCFB from the JH Center for NTM and Non-CF Bronchiectasis, and 60 healthy control participants, gender and aged-matched to the CF arm, from the Johns Hopkins Center for Immunization Research (CIR).

Study location: Study participants will be seen at the Johns Hopkins Center for Immunization Research located at 624 N Broadway Rm 117 Baltimore, MD 21205.

Study procedures:
Recruitment:
Recruitment for this study will be conducted either in person or remotely. Participants with CF and NCFB who are current patients of the Johns Hopkins Adult Cystic Fibrosis Center or the Johns Hopkins Center for Nontuberculous Mycobacteria and Bronchiectasis or other Johns Hopkins Pulmonary or Infectious Disease subspecialty clinics will be evaluated for study participation and approached for recruitment if eligible. Recruitment of healthy controls will occur through the Johns Hopkins Center for Immunization Research. Healthy controls will be gender and age-matched to within +/-5 years to the CF arm.

Potential subjects will be made aware of the study by a member of their clinical team and agree to be approached by the study team. The study team's clinical patients and individuals who have previously agreed to be contacted for future research will be approached for study participation. Study recruitment and enrollment will take place privately via telephone or through a HIPAA compliant video call system. Potential participants will be provided information about the study and will review the consent form with a study team member.

Screening:
CF and NCFB screening: HIPAA form 4 waiver has been requested in order to screen participants with CF and NCFB who are current patients at Johns Hopkins. Individuals with CF and NCFB will be screened using an EPIC chart review to assess past medical history. After a participant has been deemed eligible, he or she will be contacted by a member of the study team

via phone to confirm details of study eligibility and assess interest in study participation. Following screening, if individuals are eligible and are interested in study participation, they will be provided with a copy of the study consent form for review. If individuals with CF and NCFB decline study participation, then their reason for not participating will be recorded for future feasibility analysis regarding BCG vaccination trials in these patient populations. Information regarding which individuals have declined study participation will be retained to ensure that individuals are not contacted more than once.

Healthy volunteer participants will be screened according to the CIR protocol (JH200).

- Pre-Screening Evaluation: The purpose of the pre-screening evaluation is to determine subjects' basic eligibility to participate in the screening study. This evaluation may be completed by phone or in-person. When we contact or are contacted by an individual who has expressed an interest in participating in a research study, the pre-screening evaluation is performed using IRB-approved tools. Following an IRB-approved screening script, the individual is provided with a general overview of the screening process and he/she is informed of the types of trials conducted at the CIR. We then request the individual's permission to administer an IRB-approved pre-screening questionnaire in order to determine basic eligibility.

- Screening Visits: Adult subjects who are eligible for screening and express interest in participating in a study are scheduled for a screening visit. All potential screening procedures are described in the JH200 research plan but not all procedures will be performed on every subject. Specific screening procedures vary to minimize unnecessary procedures with considerations for subject safety and comfort, logistics as well as the current recruitment needs of the CIR. The screening study may require one or more visits of approximately 2-4 hours. Screening procedures will be performed at JHSPH facilities. Screening visit(s) will include HIV testing using enzyme-linked immunosorbent assay (ELISA), rapid antibody test and/or confirmatory test, if necessary. Healthy volunteers will also be asked to sign a Medical Release Form during the screening visit. In the event that an adverse event occurs during the course of the study, this Medical Release Form will be used to obtain relevant medical records to allow for appropriate adverse event tracking.

- Compensation for screening visits: Individuals will be paid $80 for completion of a screening visit if they are found to be eligible and enrolled in the study.

Informed Consent:
Investigators, research coordinators, and other study team members will be responsible for approaching potential research participants to seek informed consent. Informed consent will occur either in person or remotely via DocuSign (details below). Participants will be given adequate time to consider the research study and ask questions prior to signing the consent form.

Healthy volunteer participants who are deemed eligible for this study during in-person screening visits may then complete the informed consent process immediately after the screening visit.

For remote consent we intend to use DocuSign, the Institution's approved and 21 CFR Part 11-compliant software, to obtain a secure electronic signature. Once the IRB approves our consent form(s), we will use the IRB-approved consent form(s) as the base for the DocuSign template. The IRB-approved document will not be altered other than to overlay locations where

signatures, initials, dates or other DocuSign fields will be added to create the study-specific DocuSign template.

We will send the consent to the participant via DocuSign, providing a participant-specific code in advance of sending the document via DocuSign, that will be required when the participant accesses and signs the consent. The consent discussion may take place via phone or video conference (e.g. Zoom). Participants will be given adequate time to consider the research study and ask questions prior to signing the consent form. When ready to sign, the participant will enter their code, verifying that the person signing the consent is the person that we spoke with previously, and sign the consent within the DocuSign system. Once the participant has electronically signed, the study team member obtaining informed consent will be notified that the electronic form is ready for his or her signature. Once signing is completed by all parties, both the study team and the participant can download the signed consent as a PDF. The study team will also have access to the audit log and the Certificate of Completion. The study team will load the signed consent into Epic.

After the Informed Consent process is completed, the study team member will document the informed consent process and file the IRB consent document to the research record.

Procedures prior to in-person research activities during COVID-19:
Individuals will be screened for change in baseline respiratory or systemic symptoms potentially attributable to COVID-19 or recent COVID-19 exposures on both the day before (via phone) and the day of any in-person study activities. If we are unable to reach a participant via phone for COVID screening purposes the day before an in person study visit, the research staff will administer the baseline respiratory or systemic screening questions immediately upon arrival at the study visit. The study visit may be canceled and rescheduled if an individual screens positive.

Study visit 1 (day 1):

Medical History:

Inclusion and exclusion criteria will be reviewed. A detailed medical history will be obtained to record relevant comorbidities and concomitant medications (See **Appendix B - Schedule of study surveys/standardized instruments** and **Supplemental Study Documents – Medical history forms**). Vital signs (temperature, blood pressure, heart rate, respiratory rate and oxygen saturation), including height and weight will be recorded.

Patient reported outcome measures:

For participants in the CF arm, the CFQR respiratory questionnaire will be completed by the study participant and turned in to the study team.
For participants in the NCFB arm, the QOL-B and the MRC dyspnea score will be completed by the study participant and turned in to the study team. (See **Appendix B - Schedule of study surveys/standardized instruments;** Validated PRO instruments provided in **Supplemental Study Documents**).

Pregnancy testing/counseling regarding pregnancy:

For women of childbearing potential, a urine pregnancy test will be performed. Only individuals with a negative pregnancy test will be allowed to continue in the study. Pregnancy prevention counseling will be provided to recommend the avoidance of becoming pregnant in the first month following vaccination.

Research phlebotomy:

Approximately 75cc of blood will be drawn for baseline, pre-vaccination analysis **(see Appendix A schedule of sample collection**).

BCG reconstitution:

The BCG (TICE® strain; purchased from Merck) will be reconstituted by the Johns Hopkins Investigational Drug Pharmacy under a biosafety cabinet under BSL-2 conditions. BCG (TICE® strain) will be prepared using aseptic technique by adding 1mL of sterile water to one vaccine vial (contains $1x10^8$ CFU). For the purposes of this study, reconstituted BCG will be further diluted in sterile water, such that each 0.1mL of the BCG TICE® will contain an estimated 2×10^6 CFU of *M. bovis* BCG).

Intradermal BCG vaccination:

A trained research nurse from the JH CIR will perform the vaccination procedure **per study site Standard Operating Procedure (SOP) for intradermal vaccination.** Briefly a sterile syringe containing 0.1mL of the BCG TICE® with 2×10^6 CFU of *M. bovis* BCG will be injected intradermally into the deltoid region of the arm.

Symptom diary instruction:

The participant will be instructed to keep a post-vaccination diary of symptoms (see Symptom Diary in supplemental documents). The diary will be completed once weekly for the first month post-vaccination. The diary will be turned in to the study team at the one-month post-vaccination study visit.

Safety monitoring phone call (day 2):

Virtual safety monitoring:

A member from the research staff will contact the participant by phone the day after the vaccine was given to ask about any side effects from the vaccine.
If the research staff is unable to get in touch with the participant via phone for this safety monitoring phone call, the research staff will make two additional attempts to contact the participant via phone (up to a total of three contact attempts).

Safety monitoring phone call (day 7 +/-2):

Virtual safety monitoring:

A member from the research staff will telephone the participant approximately one week after the vaccine was given to ask about any side effects from the vaccine.

If the research staff is unable to get in touch with the participant via phone for this safety monitoring phone call, the research staff will make two additional attempts to contact the participant via phone (up to a total of three contact attempts).

Study visit 2 (day 30 +/- 5 days):

Interval medical history review:

Details of any acute illness (including bronchiectasis exacerbations) that have occurred since the previous in person study visit will be recorded in a written survey

(**See Appendix B - Schedule of study surveys/standardized instruments Supplemental Study Documents – Interval Medical History Form**).
Additionally, any interval changes in medical history, medications or other vaccine administration will be recorded.

Symptom diary:

The participant-completed symptom diary during the first month post-vaccination will be handed over to the study team.

Research phlebotomy:

Approximately 75cc's of blood will be drawn from the participant, processed and stored for subsequent analysis **(see Appendix A schedule of sample collection**).

Safety monitoring:

The vaccination site will be examined for each participant to ensure proper healing and evaluated for any signs of adverse reactions. A digital photograph of the vaccination site will be taken for each participant, without any identifying information included in the photograph, and will be uploaded into each participant's file that is safely stored under Virtual SAFE Desktop.

Study visit 3 (day 90 +/- 14 days):

Interval medical history review:

Details of any acute illness (including bronchiectasis exacerbations) that have occurred since the previous in person study visit will be recorded in a written survey (**See Appendix B - Schedule of study surveys/standardized instruments Supplemental Study Documents – Interval Medical History Form**).
Additionally, any interval changes in medications or other vaccine administration will be recorded.

Patient reported outcome measures:

For participants in the CF arm, the CFQR respiratory questionnaire will be completed by the study participant and turned in to the study team.
For participants in the NCFB arm, the QOL-B and MRC dyspnea score will be completed by the study participant and turned in to the study team. (See **Appendix B - Schedule of study surveys/standardized instruments**; Validated PRO instruments provided in **Supplemental Study documents**).

Research phlebotomy:

Approximately 75cc's of blood will be drawn from the participant, processed and stored for subsequent analysis **(see Appendix A schedule of sample collection**).

Safety monitoring:

The vaccination site will be examined for each participant to ensure proper healing and evaluated for any signs of adverse reactions. A digital photograph of the vaccination site will be taken for each participant, without any identifying information included in the photograph, and will be uploaded into each participant's file that is a safely stored under Virtual SAFE Desktop. If there is any concern for reaction, the photograph taken from study visit 2 will be compared to study visit 3.

During the 3-month study period, details of acute illness (including bronchiectasis exacerbations), use of antimicrobial or immunosuppressing medications will be noted for post hoc analysis.

Study visit 4 (day 180 +/- 45 days):

Study visit 4 will take place either in person or virtually via a HIPAA-approved secure video platform, such as Zoom.

Interval medical history review:

Details of any acute illness (including bronchiectasis exacerbations) that have occurred since the previous in person study visit will be recorded in a written survey (**See Appendix B - Schedule of study surveys/standardized instruments Supplemental Study Documents – Interval Medical History Form**). Additionally, any interval changes in medications or other vaccine administration will be recorded.

Safety monitoring:

The vaccination site will be examined for each participant to ensure proper healing and evaluated for any signs of adverse reactions. A digital photograph of the vaccination site will be taken for each participant, without any identifying information included in the photograph, and will be uploaded into each participant's file that is a safely stored under Virtual SAFE Desktop.

Laboratory procedures for study samples:

Study visit 1: pre-vaccination blood sampling:

Baseline testing will include formal latent TB test with T-spot IGRA, and Ag85A IGRA. Specialized analysis will include cytokine, flow, and infection assays.

Study visit 2 and 3 blood sampling:

These subsequent blood draws will be analyzed first to confirm BCG uptake. This will be done by analyzing BCG Ag-specific (including Ag85A) cytokine release using multiplex ELISA & RT-PCR as well as BCG Ag-specific T cell activation using flow cytometry with intracellular cytokine staining (ICCS). Serum cytokines will also be measured.

Blood will also be analyzed to determine if BCG vaccination elicits NTM cross-reactive immune responses in CF and NCFB by analyzing *Mycobacterium avium* (*Mav*) and *Mycobacterium abscessus* (*Mab*) Ag-specific cytokine release and T-cell activation using multiplex ELISA and RT-PCR as well as flow cytometry with ICCS.

We will then determine if BCG vaccination alters NTM killing activity in PBMCs from CF and NCFB. This will be done by infecting human monocyte-derived macrophages (MDM) with *Mav* and *Mab* followed by washing. Autologous T cells expanded with BCG for 7 d will then be added at a ratio of 10:1 and co-cultured for 72 hrs at which time cells will be detergent-lysed and *Mav* or *Mab* CFU counts determined by plating.

We will use PBMCs to assess the PBMCs from CF, NCFB, and HC pre- and post-vaccination for three key trained immunity parameters: i. pro-inflammatory cytokine release, ii. macrophage reprogramming, and iii. epigenetic changes. For pro-inflammatory cytokine release we will measure the following cytokines IFNβ, IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-10, and IL-17. For macrophage reprogramming, we will measure the following 5 populations: inflammatory macrophages, TNFα expressing M1 macrophages, IL6 expressing M1 macrophages, M2 macrophages, and IL-10 expressing M2 macrophages. For the epigenetic changes we will assess the activation mark H3K4Me3 and the repression mark H3K9Me3 in the TNFα and IL6 promoter regions by ChIP assay. We will compare the relative responsiveness of cells from each group (CF, NCFB, and HC) over time from pre-vaccination and the two post-vaccination time points. We will also compare the relative responsiveness of cells from CF and NCFB subjects to those from HCs to determine if there are measurable immune response deficiencies in CF and NCFB patients.

b. If your study involves data/biospecimens from participants enrolled under other research studies with a written consent or under a waiver of consent, please list the IRB application numbers for those studies. Please note: Certificate of Confidentiality (CoC) protections applied to the data in source studies funded by NIH or CDC will extend to this new study if the funding was active in 2016. If this situation applies, Section 36, question 4 in the application will need to be answered "Yes" and "Hopkins Faculty" should be selected in question 7. No other documents are required.

   N/A

c. Study duration and number of study visits required of research participants.

   Study duration is 6 months. Each participant will have 4 study visits including the Study day 0 visit, study day 30 (+/- 5 days), study day 90 (+/- 14 days), and study day 180 (+/- 45 days), not including screening.

d. Blinding, including justification for blinding or not blinding the trial, if applicable.

   This is a pilot study designed to test the efficacy of BCG vaccination for trained immunity against NTM infection in both CF and NCFB individuals. No blinding will be conducted. If the results from this present study are favorable, a larger, blinded, placebo-controlled trial that was powered for efficacy could be designed for either of these two infections.

e. Justification of why participants will not receive routine care or will have current therapy stopped.

   N/A. All CF and NCFB participants will continue to receive routine standard care for bronchiectasis without any interruptions.

f. Justification for inclusion of a placebo or non-treatment group.

   All participants will receive the intradermal BCG vaccination. There is no placebo or non-treatment group in this study. Following the results of this study, a larger, blinded, placebo-controlled trial may be designed.

g. Definition of treatment failure or participant removal criteria.

The goal of this study is not to achieve efficacy in prevention of infections, but rather to assess biomarkers of immune protection that are altered by BCG vaccination of vulnerable populations. Study samples will not be analyzed in real time.

Given these factors, we do not have specific criteria for participant removal. If there are unforeseen circumstances during which a participant will not be able to present for study visits 2 and 3 (research phlebotomy visits), these will be evaluated on a case by case basis by the PI.

h. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

This is a single vaccine design and there is no further booster vaccine planned. Participants will continue to receive standard of care for their medical conditions during and after study completion.

5. Inclusion/Exclusion Criteria

- **Inclusion criteria (CF/NCFB arms):**
  - Confirmed diagnosis of either CF or NCFB
  - Adult male and non pregnant females ages 18-65 (inclusive)
  - FEV1 $\geq$ 40%
  - Willingness to participate in the study after all aspects of the protocol have been explained and written informed consent obtained.
  - Available for the study duration, including all planned follow-up visits

- **Inclusion criteria (healthy controls):**
  - Adult male and non pregnant females ages 18-65 (inclusive)
  - Negative HIV enzyme-linked immunosorbent assay (ELISA), rapid antibody test and/or confirmatory test, if necessary, at screening
  - Willingness to participate in the study after all aspects of the protocol have been explained and written informed obtained
  - Available for the study duration, including all planned follow-up visits

- **Exclusion criteria (all arms):**
  - Current or prior history of active or latent TB (per report, not formally tested) or NTM infection
  - Prior BCG vaccination
  - Previous vaccine in the past 4 weeks
  - History of severe anaphylaxis to any vaccine or vaccine components
  - History of organ/bone marrow transplantation or other immunosuppressing condition, including HIV
  - Immunosuppressing drugs (including oral corticosteroids equivalent to >10mg of prednisone for 5 days) in the 30 days prior to study enrollment
  - Cirrhosis or portal hypertension
  - Pregnant or breastfeeding

- Receipt of another investigational product in the last 28 days or planned receipt during this study
- Has any other condition that, in the opinion of the principal investigator, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving study objectives

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The BCG vaccine is given intradermally to infants and children worldwide to protect against disseminated TB in most countries except the US and Canada. Its dosing for intradermal vaccination and safety profile is well established.

We have specifically chosen the BGC vaccine to test its effects on NTM because the BCG vaccine has been shown to provide some protection against diseases other than TB by boosting the immune system through trained immunity. Furthermore, based on a recent study by Abate et al there is evidence that the BCG increases protective immunity against NTM in healthy subjects[16].

Due to a current global shortage of BCG VACCINE TICE®, we have received approval from the FDA to use TICE® BCG labeled for intravesical use in this study. These products are identical in formulation, but are labeled differently to indicate different routes of administration.

For this study, the selected dose of BCG TICE® is 0.1mL (~$1x10^6$ CFU), which is lower than the package insert dose of (0.2-0.3 mL of 2-3 $x10^7$ CFU). The lower dose was chosen to be consistent with the BCG dose used in a recent study by Abate et al. that showed the BCG (TICE® strain) induced blood-based markers of protection against NTM in healthy controls[16].

In addition, we are using intradermal administration instead of the percutaneous administration that is approved by the FDA. Intradermal route has been chosen for two reasons: 1) in comparison to percutaneous administration, intradermal administration provides more standard person-to-person dosing, and 2) intradermal dosing has been shown to provide a more robust immune response compared to percutaneous administration[24].

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

TICE® BCG for intravesical use is licensed by the FDA in the US and is indicated for the treatment and prophylaxis of carcinoma in situ (CIS) of the urinary bladder, and for the prophylaxis of primary or recurrent stage Ta and/or T1 papillary tumors[22].

BCG VACCINE TICE® for percutaneous use is licensed by the FDA in the U.S. for the prevention of tuberculosis in persons not previously infected with *M. tuberculosis* who are at high risk for exposure. The Advisory Committee on Immunization Practices (ACIP) and the Advisory Committee for the Elimination of Tuberculosis recommends the BCG intradermal vaccine for the following circumstances[23]:

1. Infants and children with negative tuberculin skin tests who are (a) at high risk of intimate and prolonged exposure to persistently untreated or ineffectively treated patients with infectious pulmonary TB (b) continuously exposed to persons with infectious pulmonary tuberculosis who have bacilli resistant to isoniazid and rifampin, and the child cannot be separated from the presence of the infectious patient.
2. TB exposed health care workers in high risk settings where (a) a high percentage of TB patients are infected with *M. tuberculosis* strains that are resistant to both isoniazid and rifampin, (b) transmission of such drug resistant *M. tuberculosis* strains to health care workers and subsequent infection is likely (c) comprehensive TB infection control precautions have been implemented, but have not been successful.

Our study does not include any of these indications. The purpose of this study is to evaluate whether BCG vaccine can be effective in protection against NTM infections in patients with CF and NCFB. We have received an IND exemption from the FDA for the use of BCG in this study (See supplemental study documents).

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

See above. We have received an IND exemption from the FDA for the use of BCG in this study (see Supplemental study documents).

7. Study Statistics

a. Primary outcome variable.
- BCG Ag-specific cytokine release

b. Secondary outcome variables.
- NTM cross protectivity
- Development of lung infections among the CF/NCFB arms during the 6-month period
- Markers of trained immunity

c. Statistical plan including sample size justification and interim data analysis.

Sample Size Calculations: The primary hypothesis tests are based upon enrollment of 60 individuals within each study arm while assuming a conservative attrition and dropout rate of 10% in each study group. Due to the pilot nature of this project, participants that drop out of the study will not be replaced. Power calculations were based upon testing a mean difference in slopes between groups assuming a within-person correlation of observations of 0.1 and have assumed that baseline response will not differ between groups; preliminary data for standard deviation of markers was obtained from Abate et al. We will have 80% power to detect changes in the mean difference in slopes ranging from approximately 11% (TNFα) to 65% (IL17) for markers of BCG-stimulated immune response, NTM-induced cytokine, and NTM killing activity between the CF and control groups and between NCFB and control groups at each time point at a significance level of 0.05. Given the heterogenous responses in individuals, we consider a mean difference in slopes of 40%, indicative of an immune response deficiency, between groups to be a clinically meaningful difference.

For detecting differences in TI, we will have 80% power to detect mean differences ranging from approximately 10-70% of TI at each time point between groups at a significance level of 0.05 assuming the same conditions as above, with the exception that the time variable will be the pre/post-stimulus condition. We will repeat these analyses for each time point. We have based power calculations using Cohen's f^2 (multiple correlation/1- multiple correlation) due to the lack of available data in these populations to estimate mean differences.

Statistical Plan: Demographic and clinical characteristics of each study group will be described. Descriptive statistics, including proportions means and standard deviations and medians and interquartile ranges will be used to summarize BCG-stimulated immune response and NTM-induced cytokine and NTM killing activity at each time point (0, 30 days and 90 days) for each study group. Log-transformations of markers will be performed if distributional assumptions indicate. The primary statistical procedures will utilize linear mixed models to evaluate the mean difference in each marker from time 0 to 30 days and from time 0 to 90 days. The model takes the following form: $E[Y_{ij}] = b_0 + b_1\ time_j + b_2\ group_i + b_3\ group * time + a'z_i + \alpha_i$ where Y_{ij} is the marker of interest for subject i at time j, group denotes the CF/NCFB/control status, time is a numeric visit number, z_i can include a vector of potential confounders and α is a subject-specific random effect to account for within-person correlations of the outcome while assuming the outcomes follow a Gaussian distribution. The parameter b_3 is the parameter of interest here as it quantifies the effect of CF/NCFB status on changing markers from baseline. Importantly, this model specifically allows us to account for baseline differences between groups, if they do exist. Secondary analyses will leverage the full longitudinal data collection of each of the three time points utilizing the previously described methods.

Interim Analysis: An interim safety assessment will be conducted once 30 CF, 30 NCFB, and 30 healthy controls have been enrolled and have completed the intradermal BCG vaccination and the 30-day blood draw.

d. Early stopping rules.

If there are two or more of the same Grade 3 adverse events (severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care or ADLs), or a single Grade 4 adverse events (life threatening consequences; urgent intervention indicated), the study will be halted until the Data Safety Monitoring Board (DSMB) can meet to discuss the appropriate actions that should be taken.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Following BCG vaccination, it is expected that nearly all individuals will exhibit a positive tuberculin skin test (also referred to as a purified protein derivative test or PPD) for a period of approximately five to seven years. Thus, latent TB testing via PPD method may be confounded by BCG vaccination. However, latent TB status can still be accurately determined by diagnostics blood tests (ex: QuantiFERON-TB Gold and ELISPOT TB).

Common adverse events from intradermal BCG vaccination include pain at injection site, a local skin reaction at the site of vaccination, which may ulcerate and scar, cause regional suppurative lymphadenitis, and osteitis, a rare complication (approx. complication rate

0.39/1,000,000). The most serious complication of BCG vaccination is disseminated BCG infection. The most frequent disseminated infection is BCG osteomyelitis (0.01 to 43 cases per 1,000,000 doses). Fatal disseminated BCG infection has occurred at a rate of 0.06-1.56 cases/1,000,000 doses. These primarily have occurred in immunocompromised hosts who are excluded from participating in this study.

There are rare case reports that vaccination against other viral infections, including SARS-Co-V2 and influenza, can cause transient erythema at the site of previous BCG vaccination, with no longterm clinical consequences.

Rarely, BCG vaccination can lead to transient vaccine-induced lymphadenopathy, which may present diagnostic dilemma for women undergoing screening mammography for breast cancer.

Common adverse events from routine blood draws include mild pain at blood draw site, minor bleeding and/or bruising.

b. Steps taken to minimize the risks.

Experienced nursing and research staff from the JH CIR will perform the intradermal vaccinations and study blood draws.

To minimize concerns, we are excluding individuals with immunosuppression, children, elderly, and pregnant/breastfeeding participants.

To minimize confounding results on mammography, female participants will be advised to delay screening mammography performed for a minimum of 3 months following BCG vaccination.

We will adhere to guidance on DSMB activities (see attachment). The data and safety monitoring plan will include reporting any adverse events to the IRB and the DSMB in the quarterly progress reports. In addition, we have a detailed plan on how to deal with serious adverse events that may arise, which will include reporting to the chair of the DSMB and IRB within 24-hours of notification. The study will be conducted according to the FDA's Good Clinical Practice Guidelines.

A DSMB has been created to function as an independent group of experts who will advise the study investigators. The members of the DSMB will serve in an individual capacity and provide their expertise and recommendations. The members are not co-investigators of the study and have no undisclosed conflict of interest.

Safety Monitoring Plan:
Following BCG administration, participants will be instructed to contact the investigative team if she or he experiences symptoms such as fever of 101.5°F or greater, or acute localized inflammation persisting longer than 2-3 days, which would be suggestive of active infections. If an illness of this degree has occurred, an evaluation for a serious infectious complication will be performed which includes consultation with an infectious disease physician. In individuals who develop persistent fever or experience an acute febrile illness consistent with BCG infection,

two or more antimycobacterial agents will be administered while diagnostic evaluation, including mycobacterial blood cultures are conducted.

The DSMB will also be charged with reviewing any complaints from patients, family members, and clinicians about any aspect of the study including recruitment procedures and study implementation. All potential participants will be provided with contact information to register complaints, if they experience adverse events, coercion or other problems. Participant complaints will be reported to the IRB pursuant to Hopkins policies. The DSMB will modify or stop the study if any such complaints represent a legitimate concern about the study procedures or methods.

The DSMB has the authority to halt the study if it perceives that harm is occurring due to the intervention.

c. Plan for reporting unanticipated problems or study deviations:

All unanticipated problems or study deviations will be reported per standard Johns Hopkins IRB procedures.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

The data are being recorded on an encrypted laptop and SAFE Desktop. All files will be password protected. Only specified study team members will have access to the data. The data set will not include any sensitive information. All blood samples will be deidentified and will not be traceable back to participant.

e. Financial risks to the participants. N/A

9. Benefits

a. Description of the probable benefits for the participant and for society.

- Individual benefits: The individual may experience positive emotions from contributing to the development of patient-centered interventions by being able to share their experiences. They may also benefit from the protective nature of the BCG vaccine.
- Societal benefits: This study will provide formative information regarding the efficacy of the BCG vaccine to provide immunity towards NTM infections. These data will inform future larger studies designed to study regarding the efficacy of the BCG vaccine against NTM infections in the CF and NCFB populations.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.
   - Healthy controls will be compensated $80 for completion of the screening visit if they are found to be eligible and enrolled in the study.
   - Study participants from all arms will receive $200 for the initial visit where they will undergo a blood draw and be administered the BCG vaccine. Participants will then receive $80 for each subsequent study blood draw. Total compensation for participation in this study will be $360 (excluding compensation for screening of healthy controls).

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

- There will be no costs directed towards the participants.

Appendix A: Schedule of sample collection

Study visit	Sample collection
Screening visit (healthy volunteers only)	HIV ELISA, rapid antibody test and/or confirmatory test, if necessary
Visit 1	Urine pregnancy test Blood draw (pre-BCG vaccination). Approximately 75cc (total) of blood collected in: SST, lithium-heparin tubes, PAXgene RNA, EDTA
Visit 2 (Day 30 +/- 5)	Approximately 75cc (total) of blood: collected in: SST, lithium-heparin tubes, PAXgene RNA, EDTA
Visit 3 (Day 90 +/- 14)	Approximately 75cc (total) of blood: collected in: SST, lithium-heparin tubes, PAXgene RNA, EDTA

Appendix B: Schedule of study surveys/standardized instruments

Study visit	Healthy volunteers	CF	NCFB
Visit 1 (Day 1)	Healthy Volunteer Medical History Form	CF Medical History Form, CFQR	NCFB Medical History Form, QOL-B, MRC Dyspnea Scale
Phone call (Day 2)	Phone safety monitoring form (all arms)		
Phone call (Day 7 +/- 2)	Phone safety monitoring form (all arms)		
Visit 2 (Day 30 +/- 5)	Interval Medical History Form (all arms)		
Visit 3 (Day 90 +/- 14)	Interval Medical History Form	Interval Medical History Form, CFQR	Interval Medical History Form, QOL-B, MRC Dyspnea Scale
Visit 4 (Day 180 +/- 45)	Interval Medical History Form	Interval Medical History Form	Interval Medical History Form

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