FRED HUTCHINSON CANCER CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

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Title of Protocol:
A Phase I single site open label clinical trial for the development of a human BCG challenge model to assess TB drugs and vaccines

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FHCRC IRB Approval
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1.0 **PROTOCOL SYNOPSIS**

Protocol Title	*A Phase I single site open label clinical trial for the development of a human BCG challenge model to assess TB drugs and vaccines*
Trial Phase	*Phase 1*
Study Objectives	***Primary**:* *1. Evaluate the safety and tolerability of Intradermal Tice® BCG followed by a short course (3 days) of isoniazid (INH).* *2. Assess the BCG bacterial burden in skin biopsies as measured by culture.* ***Secondary**:* *1. Assess the BCG bacterial burden in skin biopsies as measured by a novel qPCR assay.* *2. Evaluate peripheral blood cellular frequencies (Trucount) after intradermal BCG.* *3. Evaluate adaptive immune response after intradermal BCG (validated intracellular cytokine staining after stimulation).* ***Exploratory*** *1. To conduct analyses related to furthering the understanding of TB, immunology, vaccines, and clinical trial conduct.*
Outcome Measures	***Primary Endpoints:*** *1. The rate of AE's/SAE's* *2. Colony forming units (CFU) from culture* ***Secondary Endpoints:*** *1. Quantitative bacterial 16S ribosomal DNA PCR* *2. Microbial viability testing pre-ribosomal RNA RT-PCR* *3. Humoral and cellular immune responses after BCG immunization*
Summary of Study Design	*This is phase 1, open-label, randomized clinical protocol to develop a human challenge model using the licensed and available BCG VACCINE USP (TICE® strain) with and without antibacterial Isoniazid (INH). It will involve 10 participants who will be screened and consented, given an intradermal injection of BCG; five of these participants will receive oral INH for 3 days. Participants will undergo physical exams, clinical evaluations, blood draws, urine collections, skin biopsies, and pregnancy tests. This study will measure the rate of replication by utilizing qPCR and in vitro culture, systemic innate and adaptive immune responses, including humoral and cellular assay analyses and the evaluation and PPD/IGRA status.*
Population	*10 healthy male and non-pregnant female individuals, HIV and TB uninfected, aged 18 to 45 years, with no extensive residence in high TB endemic country.*

Drugs	• *BCG VACCINE USP (TICE® strain) has been licensed for percutaneous use and is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis.* • *Isoniazid is a licensed antibacterial available as 100mg and 300mg tablets for oral administration.*
Dose	*Ten participants will receive one intradermal injection of $2x10^6$ cfu Tice® BCG (ID), and five of these participants will receive oral tablet(s): either three tablets of 100mg or one tablet of 300mg of INH each day for 3 days following the injection of BCG vaccine.*
Duration of Participation	*16 to 24 weeks (screening up to 8 weeks prior to enrollment, study follow up for 16 weeks after enrollment)*

2.0 **STATEMENT OF COMPLIANCE**

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), Office for Human Research Protections (OHRP), IRB policies of Fred Hutchinson Cancer Center, and collaborating institutions. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) (If applicable), funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

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

3.0 **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.

James Kublin, MD., MPH. Principal Investigator	Date

4.0 **LIST OF ABBREVIATIONS**

AE	Adverse Event
ALT	Alanine Aminotransferase
BCG	Bacillus Calmette-Guerin
BMI	Body Mass Index
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CRS	Clinical Research Support
DSMC	Data and Safety Monitoring Committee
EBA	Early Bactericidal Activity
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Center
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
IA	Immunoassay
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Intradermal
IEC	Independent or Institutional Ethics Committee
INH	Isoniazid
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MDR/RR-TB	Multidrug and Rifampicin-resistant Tuberculosis
MOP	Manual of Procedures
MTB	Mycobacterium Tuberculosis
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NK	Natural Killer
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
OTC	Over the Counter
PBMC	Peripheral Blood Mononuclear Cell
PoD	Prevention of Disease
PoI	Prevention of Infection
PoR	Prevention of Relapse
PPD	Purified Protein Derivative
PI	Principal Investigator
rRNA	Ribosomal Ribonucleic Acid
RT – PCR	Reverse-Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
Seattle MCTC	Seattle Malaria Clinical Trials Center
SMC	Safety Monitoring Committee
SRC	Scientific Review Committee

TB	Tuberculosis
WBC	Whole Blood Cell
WHO	World Health Organization

5.0 **BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE**

5.1 **Background Information**

5.1.1 The Challenge of Achieving TB Control

Tuberculosis (TB) can be diagnosed, treated and prevented, yet TB is the leading cause of death from an infectious agent globally, accounting for ~1.6 million deaths in 2016 - 2017[1]. It is also the leading cause of death among people living with HIV, totaling 300,000 deaths in 2016 - 2017. The World Health Organization (WHO) estimated that in 2017, 10 million people developed active tuberculosis (36% in women; 9% in people living with HIV; 72% living in Africa), causing substantial global morbidity and renewing the source of transmission[2]. Multidrug and rifampicin-resistant tuberculosis (MDR/RR-TB) remains a global health threat with an estimated 558,000 MDR/RR-TB cases in 2017[3]. There are an estimated 1.7 billion people (23% of the world's population) that are latently infected with *Mycobacterium tuberculosis* (MTB), from which cases of active TB disease arise. While the WHO has set a target for ending the TB epidemic by 2030, progress has been inadequate, with TB rates declining at a rate of 2% per year[4]. Although effective TB therapies are available, the impact on the global TB epidemic has been limited. TB treatment, particularly for multidrug resistant TB, is long, toxic, has drug-drug interactions with antiretroviral drugs and suboptimal adherence. Effective TB vaccines that prevent TB infection, disease, and recurrence are essential to eliminate TB globally, and may prove to be effective against drug susceptible and resistant forms of TB, as the genetic mutations that confer resistance are not likely to alter susceptibility to immunological activity.

5.1.2 Evaluating New Vaccines for TB

Although BCG (*Mycobacterium bovis* Bacillus Calmette-Guerin) vaccination offers some protection against TB in some populations, its efficacy is suboptimal, variable, and not adequate for disease control[5]. The correlates of protective immunity and the reason for BCG's variable and partial efficacy are unknown. Very few studies have examined the immune correlates of risk associated with BCG vaccination. Development of a more effective TB vaccine is a high priority in global health.

The complexity of TB pathogenesis and immunology has led to the identification of several different populations that could be targeted for evaluations of TB vaccines. Ultimately, the objective of a TB vaccine is prevention of microbiologically confirmed TB disease (PoD). Recently a Phase 2b study showed that M72/AS01E conferred an estimated 54% efficacy against disease among latently infected individuals[6]. This is an exciting result for the field, though with only 32 primary cases, a Phase 3 PoD study and immunological correlates analyses will be needed for validation. Prior to this study, the low incidence of TB disease spurred HVTN and TB collaborators to advocate for screening vaccine candidates based on efficacy in the prevention of infection (PoI)[7]. Due to the higher incidence of infection compared to disease, such a trial can efficiently move forward promising vaccines that elicit functional immunological responses against the pathogen, mitigating risk of a large future PoD trials. In 2018 results of the first PoI study showed that BCG re-vaccination can prevent sustained MTB infection[8], generating

renewed interest in BCG and other recombinant BCG or MTB vaccine candidates for use both in adolescent and infant populations. BCG has also been considered as a challenge agent for the purposes of evaluating TB vaccines, primarily for POD.

Patients undergoing standard treatment for active TB disease are at a much higher risk of relapse or reinfection[9]. Modeling suggests that therapeutic TB vaccines (PoR) may not only reduce recurrence, shorten treatment and decrease post treatment morbidity via immune-mediated mechanisms, but may also serve as a unique setting to discover immune correlates of protection that could be applied generally to preventive vaccines. Thus, there are several promising avenues for TB vaccine development, but stage-gating strategies for down-selecting the most promising candidates are still lacking.

5.1.3 Evaluating New Drugs for TB

The development of new drugs for TB falls far short of the pressing need. The current frontline regimen of TB drugs is fifty years old, treatment requires a minimum of six months and four different agents, involves notable risks of toxicity, and drug resistance is rising. In response, experts have called for entirely new regimens to combat the TB pandemic[10]. Despite some notable and welcome recent success with the approval by the FDA of Pretomanid Tablets in combination with bedaquiline and linezolid for the treatment of a specific type of highly treatment-resistant TB of the lungs, TB drug discovery remains severely impeded by insufficient resources and outdated approaches.

Almost universally, the first test of a new agent in TB patients involves the early bactericidal activity (EBA). In this test, a patient receives a new agent as monotherapy for 2, 7, or 14 days, and the decrease in bacterial colony forming units (CFU) in sputum is determined. While the original purpose of the assay was dose finding[11], the EBA is now employed as a gating tool to predict drug efficacy in humans. Unfortunately, the predictive power of this test is notably poor[12]. For but one example, the recently approved new TB drug bedaquiline was nearly shelved prematurely due to disappointing results in the EBA[13]. In response, field experts have called for development of “novel, more efficient screening trial designs” to expand the throughput and accuracy of phase 2 studies of anti-TB agents[11].

5.1.4 The Need for a Human Challenge Model

Human challenge studies have aided the development of drugs and vaccines for malaria, typhoid, and influenza. A study design with a defined time point for challenge creates opportunities to rigorously test the efficacy of drugs and vaccines with smaller sample sizes and controlled time points for more informative data from immunologic and biologic assays. Although human challenge infections with live fully virulent MTB have not been done and have significant health risks that would not be ethical, auxotrophic strains of MTB are being developed with properties that may solve these ethical hurdles. Preparation for these future trials will create opportunities for leading efforts of future challenge studies. In addition to testing of drugs and vaccines, human challenge models provide opportunities to intensively interrogate the host immune response. Almost all in vivo human TB studies are hampered by the limitations of an observational study design with many important unknowns including the time and extent of exposure. A human challenge model, even with a less virulent Mycobacteria would address these problems.

Previous human challenge work in the TB field employs BCG. Dr. Helen McShane at the Jenner Institute, Oxford University has already created a precedent of BCG challenge studies in animals

and humans (see below). We propose a phase 1 trial to analyze the local and systemic immune response to intradermal BCG and the effects of approved drugs with known microbicidal activity. This study design will demonstrate the capabilities of our local infrastructure and expertise in TB immunology and microbiology. The trial will illuminate the nature of local and systemic immune responses to BCG and treatment response, as well as demonstrate our local capacity for newer, more innovative study designs.

5.2 **Rationale**

We propose to develop a BCG challenge model for use in short term Phase I human trials capable of assessing the ability of TB drugs and/or vaccine-induced immune responses to impact in vivo mycobacterial replication as a method of assessing antimycobacterial agents and/or protective immunity elicited by vaccines or host-directed therapy. We are well equipped to implement efficient and successful TB drug and vaccine trials, leveraging our extensive experience with the Seattle Malaria Clinical Trials Center and our collaborations with colleagues at the University of Washington, Seattle Children's Research Institute, the Infectious Disease Research Institute, as well as TB investigators globally. This demonstration of BCG as a candidate TB challenge agent will provide our respective investigators and institutions experience and data for future work.

5.3 **Preclinical Data**

5.3.1 BCG

Preclinical work by Dr. Helen McShane at the Jenner Institute, Oxford University has served to validate the BCG challenge model in animals. They used the mouse ear as a surrogate of human skin and showed that intradermal BCG vaccination protects against subsequent intradermal BCG challenge[14]. In a separate study, they also validated this strategy in rhesus and cynomolgus macaques[15]. They were able to demonstrate a detectable vaccine effect, which was similar in magnitude to that seen in an aerosol MTB infection model. Together, these data provide support the further development of a human BCG infection model to evaluate new drugs and vaccines.

5.3.2 Isoniazid (INH)

Since its introduction in 1954, isoniazid has been a cornerstone of frontline TB treatment in humans. It has also been tested in numerous animal models of TB infection including mice[16], guinea pig[17], zebrafish[18], and nonhuman primates[19]. BCG vaccination is both safe and efficacious in conjunction with INH treatment in animal models[20, 21]. In culture, BCG is as sensitive to INH as is *M. tuberculosis*[22]. These data suggest that INH provides an excellent opportunity to test novel approaches to measuring anti-TB drug efficacy in humans.

5.3.3 Novel Pre-ribosomal RNA Assay to Assess the Viable BCG Bacterial Burden in Skin Biopsies

Mycobacterial 16S ribosomal RNA (rRNA) is a sensitive indicator of the presence of BCG but does not completely discriminate between viable and non-viable organisms. To obtain more discriminating data for this viable/non-viable distinction, testing for labile pre-rRNA was explored. Like other bacteria, *M. tuberculosis* was known to transiently express pre-rRNAs that could be measured by comparing the reverse transcription PCR (RT-PCR) vs. PCR-only assay for a pre-rRNA region of the 16S rRNA[23,24]. In vitro studies have been performed in-house (Murphy Laboratory) to hold $2x10^6$ cfu BCG in nutrient-depleted conditions (PBS), nutrient-limited conditions (human serum) or nutrient-rich conditions (7H9 broth media) for four days with or

without isoniazid. After four days, 1% of the material (intended to mimic the biopsy recovery of BCG at the administration site), was diluted into drug-free 7H9 media and monitored by pre-rRNA molecular viability testing and by OD_{600} measurements of growth. BCG held in 7H9 for the first four days and then diluted into 7H9 showed high pre-rRNA levels at the time of dilution, followed by rising OD_{600} measurements and high pre-rRNA levels indicating robust growth. BCG held in serum showed no appreciable OD_{600} change during the first four days of incubation, but did show a small, but statistically significant increase in pre-rRNA compared to static cultures or drug-killed cultures. Upon dilution, the serum-held BCG showed rising pre-rRNA levels followed by rising OD_{600} levels, indicating that the pre-rRNA molecular viability assay highlighted the viable nature of these BCG, despite no appreciable change in OD_{600} during the early time points. Thus, this data suggests that pre-rRNA molecular viability testing may shorten the time needed to identify viable bacteria in a clinical sample. Additional in vitro and pre-clinical animal studies are underway to better understand whether the serum-held BCG is an appropriate surrogate for skin-inoculated BCG and to understand the ability of pre-rRNA molecular viability testing to detect viable BCG exposed to sub-therapeutic concentrations of isoniazid or other drugs.

5.4 Clinical Data to Date

In their first human study, Dr. McShane recruited 40 healthy volunteers and performed intradermal challenge with BCG in the upper arm followed by central punch biopsies after two weeks[25]. They quantified BCG using solid culture as well as quantitative PCR. They also measured local immune changes by collecting blister fluid and performing flow cytometry. In a subsequent study, they measured the effect of pre-existing BCG immunity or MVA85A-induced immunity on BCG challenge. They found a reduction in bacterial burden among participants who previously received BCG compared to those who were BCG-naive, but did not see an effect due to MVA85A[26]. In a third study, they tested variations in the source of BCG and challenge dose[27]. Since intradermal challenge may not be physiologically relevant for a pulmonary infection, they are now testing aerosol BCG challenge in humans (ClinicalTrials.gov NCT02709278).

Another group that has published in this area is led by Dr. Dan Hoft at St. Louis University, though the focus of their efforts has been bacterial persistence and shedding. In their first study, they performed serial punch biopsies adjacent to the site of BCG vaccination (rather than in the center of the lesion) in order to quantify mycobacterial shedding using PCR and solid culture[28]. More recently, they repeated this study using swabs to measure shedding non-invasively by three different methods (solid culture, liquid culture, and quantitative PCR)[29].

Together, these studies validate the feasibility and safety of our proposed study.

5.5 Protocol Agents

- *BCG vaccine (Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis):* one intradermal injection of on-label dose of 2x106 cfu Tice® BCG
- *Isoniazid (INH)*: oral tablets of on-label standard dose of either three 100mg tablets (300mg) or one 300mg tablet for 3 days post BCG injection.

5.6 Handling, Storage and Accountability and Preparation

Handling, storage and preparation will be conducted according to the instructions per the drug package inserts. The pharmacy and research team will adhere to their internal MOPs that determine accountability procedures.

5.7 **Study Compliance**

Upon enrollment, participants will be trained on the protocol procedures and receive ongoing education by study staff to encourage adherence to the protocol. There is some flexibility in the timing of clinic visits to increase protocol adherence and maximize chance of retention.

5.8 **Potential Risks and Benefits**

5.8.1 Known Potential Risks

Risks of BCG

The BCG vaccine continues to be the most widely distributed vaccine worldwide, generally provided to infants immediately after birth, so it is safe and well tolerated. Typically, most people will develop a small swelling (nodule) at the injection site that break open, release pus or blood-tinged discharge, and form an ulcer the size of a dime. The ulcer will heal over a period of weeks and forms a scar that remains for life. Occasionally, lymph nodes around the vaccination site can become tender to touch. "Flu-like" symptoms that are common to many vaccines includes fevers, chills, loss of appetite, muscle aches, and generally not feeling well can last 1-2 days.

A very small number of people (1 in 4 million) have an immediate allergic reaction to BCG. Symptoms include rash, swelling around the mouth, difficulty breathing. These can be treated with emergency medications. If severe, they can cause death.

There is a risk that by receiving the BCG vaccine, participants may develop a false positive tuberculin skin test (PPD) that will complicate the early diagnosis and treatment of latent (inactive) TB. However, a diagnosis of latent TB can be confirmed by other methods, including a clinical examination, chest x-ray, and blood test (IFN-g release assay).

Risks of Isoniazid (INH)

Participants who are administered Isoniazid may experience some side effects that include: numbness and tingling in the extremities, hepatitis (symptoms include loss of appetite, nausea, vomiting, fatigue, malaise, and weakness), nausea, vomiting, upset stomach, fever, and rash.

Participants will be closely monitored by study staff and necessary treatment will be administered to alleviate symptoms that may occur.

Risks of Intradermal Injection

Participants may experience: pain, swelling, redness, itching caused by the ID injection. There is potential for an infection at the ID challenge site.

Risks of Skin Biopsy

Participants may experience: pain, swelling, redness, bleeding and bruising at the biopsy site during and a day or two after, damage to the skin such as artery or nerve and possible scarring. There is potential for an infection at biopsy site or allergic reaction to the anesthetic or gel foam

used to close the biopsy site. Participants cannot swim in a chlorinated water until the gel foam has sufficiently closed the biopsy area.

Risks of Blood Draws
Participants may experience: pain, swelling, redness, caused by the insertion of the needle, nausea, light headedness and there is a slight chance of fainting. There is potential for an infection at the needle insertion site

Risks of PPD Skin Test
Participants may experience: pain, swelling, redness, itching caused by the insertion of the needle and/or PPD agent. There is potential for an allergic reaction to the agent and/or infection at the PPD test site.

Risk of the loss of confidentiality
There is a potential for the loss of confidentiality. Each member of the research team has completed human subjects protections, and HIPAA and confidentiality training. Trainings inform the research team members to follow confidentiality policies and best practices when handling identifiable information.

5.8.2 Known Potential Benefits

There will be no direct benefit to participants in this study. Others in the future may benefit from the information gained from this research, which will help scientists develop a better TB vaccine.

6.0 **OVERVIEW OF CLINICAL TRIAL**

6.1 **Study Objectives**

6.1.1 Primary Objectives

- Evaluate the safety and tolerability of Intradermal Tice® BCG followed by a short course (3 days) of isoniazid (INH).
- Assess the BCG bacterial burden in skin biopsies as measured by culture.

6.1.2 Secondary Objectives

- Assess the BCG bacterial burden in skin biopsies as measured by a novel qPCR assay.
- Evaluate peripheral blood cellular frequencies (Trucount) after intradermal BCG.
- Evaluate adaptive immune response after intradermal BCG (validated intracellular cytokine staining after stimulation).

6.1.3 Exploratory Objectives

- To conduct analyses related to furthering the understanding of TB, immunology, vaccines, and clinical trial conduct.

6.2 **Study Design**

This Phase I single site open-label clinical trial will evaluate the use of Tice® BCG in healthy adult participants as a challenge model for future assessment of TB drugs. Subjects (N=10) will be divided into two groups, where group 1 will receive INH in the dose of 300 mg for three days post BCG injection and group 2 (control arm) will not receive any treatment. All groups will receive a dose titration of $2x10^6$ cfu of Tice® BCG intradermally as noted in the following table:

	Group 1	*Group 2*
N	*5*	*5*
Tice® BCG Dose	*$2x10^6$ cfu*	*$2x10^6$ cfu*
INH Dose	*300 mg/3 days*	*Control*

Participants will have follow-up clinic visits that will include: clinical assessments, AE/SAE review, ID challenge assessments, blood draws for immunogenicity and mycobacteria assays, skin biopsies, general exams and PPD skin testing.

7.0 **STUDY POPULATION**

Ten healthy, HIV and TB uninfected, male and non-pregnant female subjects, 18 to 45 years old, inclusive, will be enrolled from a single study site in Seattle, Washington. The target population should reflect the Seattle King County community. Recruitment materials may be dispersed in the community and participants from other research registries may be contacted. Enrollment will occur over a 2-month period. Consent will be required for enrollment.

8.0 **SUBJECT ELIGIBLITY**

Inclusion and Exclusion Criteria must be assessed by a medically licensed study clinician to make medical diagnoses.

8.1 **Inclusion Criteria**

Participants must meet all of the following criteria to participate in this study.

1. Provide written informed consent prior to initiation of any study procedures,
2. Are males or non-pregnant females between the ages of 18 and 45 years, inclusive,
3. Women of childbearing potential* in sexual relationships with men must use an acceptable method of preventing conception** from 30 days prior to 3 months after Tice® BCG administration,
   **Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or < 1 year of the last menses if menopausal).*
   ***Includes, but is not limited to, sexual abstinence, monogamous relationship with vasectomized partner who has been vasectomized for 6 months or more prior to the subject receiving Tice® BCG, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing ®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").*

4. For women of childbearing potential, negative serum pregnancy test at screening and negative urine pregnancy test within 24 hours prior to enrollment and Tice® BCG administration,
5. Are in good health, as judged by the investigator and determined by vital signs (oral temperature, pulse, and blood pressure), medical history and physical examination,
6. Have a negative HIV-1 ELISA test,
7. Have negative serology tests for hepatitis B surface antigen and hepatitis C virus antibody,
8. Have a negative QuantiFERON-TB Gold test,
9. Negative is defined as Nil response < 0.8 IU/ml and TB Antigen response minus Nil response <0.35 IU/mL or TB Antigen response minus Nil response > 0.35 IU/mL and < 25% of Nil response and Mitogen response minus Nil response > 0.5 IU/ml,
10. Have a urine dipstick for protein less than 1,
11. Have a urine dipstick negative for glucose,
12. Ability to understand and complete all study visits as required per protocol and be reachable by telephone.

8.2 **Exclusion Criteria**

Participant cannot meet any of the following criteria and must be excluded from participating in this study.

1. Have a history of suspected, confirmed, treated or have other evidence of active tuberculosis, Symptoms may include recurrent fever, fatigue, night sweats, weight loss, oral ulcers, diarrhea, nausea, vomiting, or bleeding,
2. Have any systemic symptoms* within 72 hours before Tice BCG administration or signs of lymphadenopathy, hepatosplenomegaly, or pulmonary disease by physical examination on day of Tice BCG administration,
   *Includes fever, chills, malaise, fatigue, headache, night sweats, weight loss, nausea, vomiting, bleeding, diarrhea, abdominal pain, rhinorrhea, cough, wheezing, or shortness of breath.
3. Have history of any significant acute or chronic medical conditions* or need for chronic medications that, in the opinion of the investigator, will interfere with immunity or affect safety,
   **Includes, but is not limited to, disorders of the liver, kidney, lung, heart, or nervous system, or other metabolic or autoimmune/inflammatory conditions. Have any history of excessive scarring or keloid formation.*
4. Have household contact or occupation involving significant contact with someone who is immunocompromised,
   **Includes persons with HIV, AIDs, or active cancer; infants (children < 1 year); pregnant women; or persons who are immunosuppressed for approximately 6 weeks (during the time of active ID lesion drainage).*
5. Have a history of epilepsy (does not include febrile seizures as a child),
6. Have a pacemaker, prosthetic valve, or implantable cardiac devices,
7. Have a history of bleeding disorder,
8. Have a known allergy to any Tice BCG components (glycerin, asparagine, citric acid, potassium phosphate, magnesium sulfate, iron ammonium citrate, and lactose),
9. Received blood products or immunoglobulin within 6 months prior to Tice BCG administration,
10. Received immunotherapy within one year prior to Tice BCG administration,
11. Received or plan to receive live attenuated vaccines 4 weeks before or after Tice BCG administration,
12. Received or plan to receive inactivated or killed vaccines 2 weeks before or after Tice BCG administration,

13. Plans to enroll in another clinical trial* that could interfere with safety assessment of the investigational product at any time during the study period,
    **Includes trials that have a study intervention such as a drug, biologic, or device.*
14. Received an experimental agent* within 30 days prior to Tice BCG administration or planned receipt of an experimental agent within 90 days after Tice BCG administration,
    **Includes vaccine, drug, biologic, device, blood product, or medication.*
15. Have a history of use of a systemic antibiotic within 14 days prior to Tice BCG administration or planned use of a systemic antibiotic for 3 months after Tice BCG administration,
16. Have any medical, psychiatric, occupational, or behavioral problems that make it unlikely for the subject to comply with the protocol as determined by the investigator,
17. Are health care providers at the highest risk of acquiring MTB infection, such as pulmonologists performing bronchoscopies on TB patients,
18. Are breastfeeding or plan to breastfeed at any given time throughout the study,
19. Have long term use* of high dose oral or parenteral glucocorticoids**, or high-dose inhaled steroids***.
    **Defined as taken for 2 weeks or more in total at any time during the past 2 months.*
    ***High dose defined as prednisone ≥ 20 mg total daily dose, or equivalent dose of other glucocorticoids.*
    ****High dose defined as > 800 mcg/day of beclomethasone dipropionate or equivalent. If short term corticosteroids are given, then the subject should not receive Tice® BCG or have blood collected for immunogenicity studies within 1 week of steroid administration.*
20. Have immunosuppression or are taking systemic immunosuppressants as a result of an underlying illness or treatment,
21. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 36 months prior to Tice® BCG administration,
22. Any active neoplastic disease,
23. Have a pulse rate less than 50 bpm or greater than 100 bpm,
24. Have a systolic blood pressure less than 90 mm Hg or greater than 140 mm Hg,
25. Have a diastolic blood pressure less than 50 mm Hg or greater than 90 mmHg,
26. Have a WBC less than $4.0x10^3$/uL or greater than $10.5x10^3$/uL,
27. Have hemoglobin less than $11.5x10^3$/uL (female) or less than $12.5x10^3$/uL (male),
28. Have a platelet count less than $140x10^3$/UL,
29. Have a creatinine greater than 1.30 mg/dL,
30. Have an ALT (SGPT) greater than 40 IU/L (female) or greater than 55 IU/L (male),
31. Have known HIV, Hepatitis B, or Hepatitis C infection,
32. Have a history of alcohol or drug abuse in the last 5 years,
33. Have had a positive PPD skin test in the past or received BCG vaccine (BCG vaccination history will be determined by self-report, country of birth, and/or evidence of BCG scar),
34. Have a BMI >35,
35. PPD skin test within 2 months prior to Tice BCG administration or planned receipt during the study other than from participation in this study,
36. Oral temperature ≥ 100.4°F (≥ 38.0°C) or other symptoms of an acute illness within 3 days before Tice BCG administration. (Subject may be rescheduled),
37. Any medical disease or condition that, in the opinion of the investigator, is a contraindication to study participation*,
    **Includes medical disease or condition that would place the subject at an unacceptable risk of injury, render them unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or their successful completion of the study.*

38. Have any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol or compromise the interpretation of data or the scientific integrity of the protocol.

9.0 **RECRUITMENT**

Participants will be recruited from the Seattle King County area. Recruitment materials may be posted in community areas such as, but not limited to: the campuses of Fred Hutchinson and University of Washington, and community businesses that allow the posting of flyers. Enrollment may also come from participants of other research studies and registries.

All recruitment methods and will be reviewed and approved by the IRB prior to approaching participants. The participants from other studies or repositories must have granted consent to allow being contacted and given information about other research studies. The method of contact is determined by the IRB's approval per the other research studies protocols and registries as they may have certain limitations.

The gender and ethnic and racial demographics will reflect the Seattle King County community at large.

10.0 **STUDY PROCEDURES AND EVALUATIONS**

10.1 **Consent**

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout their study participation. The consent process will include the explanation of the research study to the participant and answer any questions that may arise. The study team member who conducts the consent process will be medically qualified to answer any health concerns, risks and possible benefits of participation in this study. The consent form will be provided to the subject which they may share with their families. The consent form describes in detail the study procedures and risks. The participant will be given an adequate amount of time to review the consent form prior to giving consent by signing the form. Consent forms are IRB reviewed and approved prior to being distributed to participants. They are written documentation of informed consent and the participant is required to sign it prior to enrolling in the study. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants for their records.

10.2 **Screening**

Those who consent will be considered for eligibility and will undergo evaluations and assessments (screening process). Screening procedures include review of the participant's medical history and concomitant medications, and collecting their vital signs (oral temperature, pulse, blood pressure) and height and weight (to calculate BMI). The Participant will undergo a physical examination within 42 days prior to receiving Tice® BCG. Female participants of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to enrollment and receipt of Tice® BCG. Participants must have a urine dipstick for protein < 1 and negative for glucose at

screening. Participants will have their blood drawn via venipuncture to be screened for HIV, hepatitis B surface antigen, antibody to hepatitis C virus, and QuantiFERON®-TB Gold Test. Results of these screening tests must be negative.

10.3 Group Assignment

After the participant meets enrollment criteria, they are randomly assigned by a computer program to 1 of the 2 groups. In both groups, participants will be immunized intradermally with a single dose of $2x10^6$ cfu Tice® BCG.

- Group 1 subjects will receive INH dosages on Days 4, 5, and 6.
- Group 2 (control arm) will not receive INH.

10.4 Study Drug Administration

Tice® BCG and INH preparations will be performed by the participating clinical study site pharmacist and administration will be performed by a study clinician licensed to administer study product.

10.4.1 BCG injection (all participants)

One intradermal injection of Administration of BCG $2x10^6$ cfu Tice® BCG in the deltoid region, Following administration of Tice® BCG, intradermal (ID) site reactions will be assessed for at least 30 minutes.

10.4.2 INH (Group 1 only)

For the 5 participants assigned to Group 1, they will take INH: oral tablets of either three 100mg tablets (300mg) or one 300mg tablet on days 4, 5, and 6 post BCG dosing.

10.5 Study Duration

The participants will come to the study clinic 17 times to undergo study procedures. Additional visits may be required if they experience an abnormal ID challenge site. The maximum study duration will be 22 weeks (about 6 months). A screening visit, BCG dosing visit and 15 follow up visits within 4 months will occur after skin biopsy.

10.6 Evaluations

10.6.1 Physical Examination and Clinical Assessments

A physical examination will be performed within 42 days prior to getting BCG and at the final study visit. Participants medical history, concomitant medications, their vital signs (oral temperature, pulse, blood pressure), and height and weight (to calculate Body Mass Index (BMI)) will be collected. Participants will be assessed for lymphadenopathy if indicated based on review of interim medical history and clinical assessment. A medically licensed research team member will review the participants health status and eligibility at the clinic visits.

10.6.2 Participant Self-monitoring AEs/SAEs and Health Status Records

Participants will be asked to record their solicited local and systemic reactions, any unsolicited AEs/SAEs (pain, tenderness and drainage) and concomitant medications throughout the study.

They can optionally record their oral temperature as well. They will be sent a redcap survey to record this information. They will be instructed on how to measure and record AEs/SAEs.

10.6.3 Concomitant Medication and Supportive Care Guidelines

At each study visit/contact, the investigator will ask the participants about any medication taken, including herbals, vitamins and holistic/naturopathic medications, concomitant medication, including vaccines, and any other medication relevant to the protocol, including any specifically contraindicated will be recorded in the eCRF device with trade name and/or generic name of the medication, medical indication, start and end dates of treatment. Concomitant medications will include but are not limited to:

- Antibiotics,
- Over the counter (OTC)
- Prescription meds.

10.6.4 Laboratory Evaluations/Assays

Whole blood samples will be collected via venipuncture for clinical safety laboratory evaluations and BCG response assays. A total of ~730mls (~3 cups) will be drawn for the entire study. At the screening visit (visit 1)and Days 0 (visit 2), 1 (visit 3), 2 (visit 4), 3 (visit 5), 4 (visit 6), 8±1 (visit 7), 15±1 (visit 8) , 28±1 (visit 9), 56±2 (visit 10), 84±3 (visit 11), 98±1 (visit 12), 112±1 (visit 14), and 114±1 (visit 15), these standard blood draws will be collected from each participant to perform immunogenicity and humoral assays.

Clinical safety assessments

Blood samples for clinical safety laboratory evaluations (WBC, hemoglobin, platelet count, ALT (SGPT), creatinine) will be collected from each subject prior to administration of Tice® BCG at the screening visit (visit 1). These parameters must meet protocol-defined eligibility criteria to be enrolled and receive Tice® BCG. Additional clinical safety laboratory evaluations may be deemed necessary during the study.

Measuring BCG response

Skin biopsy (via XX)

Skin biopsies will be taken to quantify BCG bacteria. The ID challenge site will be biopsied on Days 3, 8, 15 and 56 (visits 5, 9, 10, and 12) after Tice® BCG administration. A 4mm skin biopsy using the punch method will be taken at the ID challenge site. A local anesthetic injection will be given to numb the site. Gel foam will be used to close the biopsy area. A medically licensed research team member will perform the biopsy.

The number of viable BCG bacteria will be measured on Day 3 from the skin biopsy by 1) quantitative CFU plating, 2) the numbers of genomes of BCG by quantitative real time PCR, and if sufficient material is available, by 3) additional assays may be performed to assess the integrity of the immune system.

Blood samples (via venipuncture)

- Serum will be collected on Days: 0, 1, 2, 3, 4, 8±1, 15±1, 28±1 56±2, and 84±3, scheduled visits will be collected for inflammatory markers (IL-2, IL-4, IL-5, IL-8, IL-10, INFγ, 1l-12p70, 1l-17A, IL-1, IL-6 and TNF-α levels) using Cytokine BeadArray, and clinical testing for acute phase reactants (ESR and CRP).

- Blood will be collected for ELISPOT/ICS and additional assays to assess the integrity of the immune system on Days: 0, 8±1, 28±1, 56±2, 84±3, and 114±1. Whole blood will be collected for RNA seq studies on Days: 0, 1, 2, 3, 4, 8±1, 15±1, 28±1, 56±2, and 84±3. Plasma obtained from the density gradients used to purify PBMCs will be collected and frozen on Days: 0, 1, 8±1, 15±1, 28±1, 56±2, 84±3, and 114±1. Anticoagulated whole blood will be collected for dendritic cell (DC), monocyte and natural killer (NK) cell assays on Days: 1, 4, 8±1, 15±1, 28±1, 56±2, and 114±1.

10.7 **Photographs**

Photographs of the ID challenge site will be taken at each follow-up visit after Tice® BCG administration starting on Day 4 through Day 56±2. If the participant has any abnormality and does not have a well-healed scar, or the site is open and/or draining, photographs will continue once/week from the day the abnormality was identified until it has resolved. All subjects will have a final photograph of the ID challenge site on Day 114±1 or upon early termination.

10.8 **PPD Skin Test**

2-Step PPD skin test will be performed and/or accessed on Days: 98±1, 100±1, 112±1 and 114±1. If the first PPD skin test result is negative, then the second PPD skin test will be performed 2-4 weeks later. If either of the 2-Step PPD skin test results is positive, defined as ≥ 10 mm of induration, then the subject will have a QuantiFERON®-TB Gold Test performed to determine TB infection or BCG immunity.

10.9 **Memory Aid eCRF Device and AE/SAE Recording Training**

Participants will be instructed on how to use the initial and on-going subject memory aids eCRF device and how to measure and record AEs/SAEs, concomitant medication and other health status information.

11.0 **STUDY SCHEDULE**

11.1 **Participant Procedures Schedule**

See Appendix B

11.2 **Clinic Visit Schedule**

<u>Screening visit: A single visit between Days -42 and -1 (visit 1)</u>

- Informed Consent
- Review medical history,
- Review concomitant medications,
- Collect vital signs (oral temperature, pulse, blood pressure),
- Collect height and weight (to calculate BMI),
- General examination,
- Collect urine for dipstick for protein < 1 and negative for glucose and chlamydia/gonorrhea test,

- Blood draw (29mls (6 teaspoons (tsp)) for testing HIV, hepatitis B surface antigen, antibody to hepatitis C virus, syphilis and QuantiFERON®-TB Gold Test. Results of these screening tests must be negative,
- Pregnancy tests for females of childbearing potential (must be negative for eligibility).
    - Serum pregnancy test at screening,
    - Urine pregnancy test within 24 hours prior to enrollment and receipt of Tice® BCG and INH.

Day 0 (clinic visit 2)

- Administration of BCG $2x10^6$ cfu Tice® BCG: one intradermal injection in the deltoid region,
  * Following administration of Tice® BCG, intradermal (ID) site reactions will be assessed for at least 30 minutes.
- Blood draw via venipuncture for immunogenicity and mycobacteria assays: 114.5mls (8 tbs),
- Participants will be instructed on how to use the initial and on-going subject memory aids eCRF device and how to measure and record AEs/SAEs prior to discharge from the clinic,
- In addition, subjects will be instructed to keep the ID challenge site covered with a dressing (sterile gauze, etc.) and all dressings that have been used to cover the ID challenge site will be collected in sealable clear plastic bags. These materials will be collected from subjects at follow-up visits and properly disposed of as biohazard waste. Subjects will also be counseled to avoid any direct contact of drainage with an immunosuppressed person.

Day 1 (clinic visit 3), Day 2 (clinic visit 4), and Day 3 (clinic visit 5)

- Assess ID challenge site,
- Day 3 (clinic visit 5) only: skin biopsy,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Blood draws via venipuncture for immunogenicity and mycobacteria assays:
    - Day 1: 6.5mls (1.5 tsp),
    - Day 2: 6.5mls (1.5 tsp),
    - Day 3: 6.5mls (1.5 tsp),
- Subjects will be reminded to record self-assessments on their redcap survey:
    - Oral temperature (optional)
    - Solicited local and systemic reactions recorded Days 1-114,
    - Unsolicited AEs/SAEs recorded Days 1-114,
    - Concomitant medications recorded Days 1-114.
- Collection of used dressing/gauze.

Days 4 (clinic visit 6), 5 (clinic visit 7), 6 (clinic visit 8), 8±1 (clinic visit 9), 15±1 (clinic visit 10), and 28 ± 1 (clinic visit 11)

- INH dosing days 4, 5, and 6 only: For the 5 participants assigned to Group 1, they will take INH: oral tablets of either three 100mg tablets (total 300mg) or one 300mg tablet for 3 days post BCG dosing,
- Days 8±1 (clinic visit 9) and 15±1 (clinic visit 10) only: Repeat skin biopsy,
- Assess ID challenge site,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- Assess for lymphadenopathy if indicated based on review of interim medical history and clinical assessment,

- Targeted physical exam and clinical assessment may be performed if indicated based on review of interim medical history, reactogenicity (as applicable),
- Blood draws via venipuncture for immunogenicity and mycobacteria assays:
    - Day 4: 6.5mls (1.5 tsp),
    - Day 8±1: 74.5mls (5 tablespoons (tbs)),
    - Day 15±1: 56.5mls (4 tbs),
      Day 28±1: 94.5mls (6 tbs),
- Collect used dressing/gauze,
- Photograph of ID challenge site.

Days 16-114±1

- On-going participant self-evaluations that will be recorded on their memory aid eCRF device for pain, tenderness and drainage. Participants can record their temperature if taken.

Days 56±2 (clinic visit 12) and 84±3 (clinic visit 13)

- Day 56±2 (clinic visit 12) only: Repeat skin biopsy
- Assess ID challenge site (if an abnormal BCG injection site is identified see Abnormal BCG Injection Site Procedures in section 11.3),
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- Assessments for lymphadenopathy if indicated based on review of interim medical history and clinical assessment,
- Targeted general exam and clinical assessment may be performed if indicated based on review of interim medical history, reactogenicity (as applicable),
- Blood draws via venipuncture for immunogenicity and mycobacteria assays:
    - Day 56±2: 94.5mls (6 tbs)
    - Day 84±3: 94.5mls (6 tbs)
- Collect used dressing/gauze,
- Photograph of ID challenge site.

Day 98±1 (clinic visit 14)

- Assess ID challenge site,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- Blood draw via venipuncture for diagnostic assays: 4mls (1 tsp),
- PPD skin test.

Day 100±1 (clinic visit 15)

- Assess ID challenge site,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- PPD skin test assessment.
  *Day 120/134: If the first PPD skin test result is negative, then the second PPD skin test will be performed 2-4 weeks later. If either of the 2-Step PPD skin test results is positive, defined as ≥ 10

mm of induration, then the participant will have a QuantiFERON®-TB Gold Test performed to determine TB infection or BCG immunity.

Days 112±1 (clinic visit 16)

- Assess ID challenge site,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- Blood draw via venipuncture for diagnostic assays: 4mls (1 tsp),
- PPD skin test.

Day 114±1 (clinic visit 17) **End of Study**

- General exam,
- Assess ID challenge site,
- Review health status and AEs/SAEs that were recorded on the memory aid eCRF device,
- Review concomitant medications,
- Blood draw via venipuncture for immunogenicity and humoral assays: 138mls (9.5 tbs),
- Photograph of ID challenge site,
- PPD skin test assessment,
- Participants returns memory aid eCRF devices.

11.3 Abnormal BCG Injection Site Procedures

The BCG injection site evaluations are scheduled from Day 0 to 84±3. However, signs of an abnormal injection site may present around Day 28. If an injection site does not have a well healed scar, or has an open and draining ulcer, then the subject will be asked to return to the clinic twice per/week from the time the abnormality is identified until it has resolved. At each of these visits the following evaluations will be completed: assessments of the ID challenge site, assessments for lymphadenopathy if indicated based on review of interim medical history and clinical assessment, as well as review AEs/SAEs, concomitant medications, and health status, and collect and dispose of returned biohazard materials. Photographs will be taken once per/week from the day of the abnormal assessment until it has resolved.

11.4 Withdrawal Procedures

Participants who discontinue from the study will be asked to complete visits 14-17. Visits 14-17 will occur using the same chronology as in the procedures schedule. If a female becomes pregnant, PBMC will not be collected on visit 17. However, PBMC will be collected from all other participants. If the participant refuses to complete visits 14-17, they will be asked to complete visit 17 which should occur at least 7 days, but ≤ 30 days, after the dose of BCG.

Procedures to be performed on visits 14-17: see above in section 11.2 Clinic Visit Schedule.

12.0 LABORATORY TESTING AND ASSAY SCHEDULE

See Appendix A for Laboratory Procedures Table.

13.0 SAFETY MONITORING COMMITTEE

A Safety Monitoring Committee (SMC) will be convened periodically by Fred Hutch to review participant safety data, which may include solicited and unsolicited AEs/SAEs, concomitant medications, clinical laboratory values and any physical examinations. Interim statistical reports may be generated as deemed necessary. The SMC may receive data in aggregate and presented by group. As an outcome of each review, the SMC will make a recommendation at that time as to the advisability of proceeding with Tice® BCG administration, and to continue, modify or terminate the study.

13.1 Primary Endpoint

- The rate of AE's/SAE's related to Tice® BCG administration reported at any point during the study,
- Summary of the distribution of viable BCG bacteria from intradermal challenge sites at 4 time points (approximately 3 days and 1, 2, and 4 weeks following Tice® BCG administration), as detected by quantitative CFU plating. The distribution will be summarized in terms of its central tendency (mean or GM) and its precision represented by the appropriate 95% confidence interval.

13.2 Secondary Endpoint

- Quantitative bacterial 16S ribosomal DNA PCR,
- Microbial viability testing pre-ribosomal RNA RT-PCR,
- Humoral and cellular immune responses after BCG immunization.

14.0 SUBJECT DISCONTINUATION OF ACTIVE TREATMENT

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

A subject may be withdrawn from the study for the following reasons:

- The subject withdraws consent,
- The subject develops a medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, would interfere with the subject's successful completion of this study, or would interfere with the evaluation of responses,
- The subject fails to comply with the scheduled study visits,
- The study is terminated,
- Any reason that, in the opinion of the investigator, precludes the subject's continued participation in the study,
- The subject becomes pregnant.

15.0 ASSESSMENT OF SAFETY

15.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Tice® BCG-related serious adverse events occurring throughout the course of the study,
2. Solicited Adverse Events – reactogenicity events following Tice® BCG administration:
    a) Local reactions including pain, tenderness, erythema/redness, and induration/swelling at the injection site.
    b) Systemic reactions including fever, chills, malaise/fatigue, headache, night sweats, nausea, vomiting, diarrhea, rhinorrhea, cough, and wheezing or shortness of breath.
3. Lesion characterized as erythematous macule, non-erythematous papule, erythematous papule, vesicle, pustule, ulcer, eschar/crust, healed, scar, or keloid,
4. Lymphadenopathy,
5. Clinical safety laboratory adverse events to include WBC, hemoglobin, and platelet count, creatinine and ALT (SGPT),
6. Unsolicited Adverse Events.
    a) Non-serious adverse events occurring in the 56 days following Tice® BCG administration.

15.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

15.2.1 Adverse Events

Adverse Event (AE):

International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study treatment recipient presenting for medical care, or upon review by a study monitor.

All AEs, including local and systemic reactions, not meeting the protocol-defined criteria for serious adverse events (SAEs) should be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited AEs includes event description, date of onset, study clinician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis), date of resolution/stabilization of the event, seriousness and outcome. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

Severity of Event:

All AEs will be assessed by the site principal investigator or appropriate sub-investigator using the protocol-defined grading system (Section 15.2.2-15.2.3). For events not included in the protocol defined grading system, the following guidelines will be used to quantify severity:

- *Mild (Grade 1)*: events require minimal or no treatment and do not interfere with the patient's daily activities.
- *Moderate (Grade 2)*: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- *Severe (Grade 3)*: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Products:

The study clinician's assessment of the relationship of an AE's relationship to study product 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 study product assessed using the following terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- *Related*: There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- *Not Related*: There is not a reasonable possibility that the administration of the study product caused the event.

15.2.2 Reactogenicity

Reactogenicity events are AEs that are known to occur with Tice® BCG. Reactogenicity events will be analyzed according to the following grading scales:

Table 1: Reactogenicity Events Grading Scales

Local Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Pain – experienced without touching the injection site	Subject is aware of pain but it does not interfere with daily activity **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires use of pain medication	Subject is aware of pain **and** it prevents daily activity
Tenderness – hurts only when injection site is touched	The area immediately surrounding the injection site hurts only when touched **and** it does **not**	The area immediately surrounding the injection site hurts when touched **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched **and** it prevents daily activity

	interfere with daily activity		
Erythema/Redness*	Does not interfere with activity	Interferes with activity	Prevents daily activity
Induration/ Swelling*	Does not interfere with activity	Interferes with activity	Prevents daily activity
Drainage	Continuous use of bandage cover or less than 2, 2x2 gauze pads/day	Needs 2-4, 2x2 gauze pads/day	Needs more than 4, 2x2 gauze pads/day

* Will be also measured in mm but size will not be used as halting criteria.

Erythema/redness, induration/swelling and lesion as analyzed by measurement will be graded as follows:

Table 2: Erythema/Redness, Induration/Swelling and Lesion Measurement Grading Scales

	Small	**Medium**	**Large**
Total Erythema/Redness*	<20 mm	20-50 mm	>50 mm
Maximum Diameter of Induration/Swelling*	<20 mm	20-50 mm	>50 mm
Central Lesion	<10 mm	10-30 mm	>30 mm

* Measurement will not be used as halting criteria

Table 3: Reactogenicity Events Grading Scales (Continued)

Systemic (Subjective)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Chills	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Malaise/Fatigue	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Headache	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Night Sweats	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Nausea	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Vomiting	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Diarrhea	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Rhinorrhea	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Cough	No interference with activity	Some interference with activity	Significant interference, prevents daily activity

Wheezing/Shortness of Breath	No interference with activity	Some interference with activity	Significant interference, prevents daily activity

Vital signs[#] (oral temperature, pulse and blood pressure) will be analyzed according to the following grading scales:

Table 4: Vital Signs Grading Scales

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever*	38.0-38.4°C 100.4-101.1°F	38.5-38.9°C 101.2-102°F	≥39°C ≥102.1°F
Tachycardia - beats per minute[†]	101-115	116-130	>130
Bradycardia - beats per minute	45-49	40-44	<40
Hypertension (systolic) mm Hg	141-150	151-155	>155
Hypertension (diastolic) mm Hg	91-95	96-100	>100
Hypotension (systolic) mm Hg	85-89	80-84	<80
Hypotension (diastolic) mm Hg	45-49	40-44	<40

[#] Vital Signs assessed at Day 1 will be considered as baseline.
* Oral temperature, no recent hot or cold beverages or smoking. Note: A fever can be considered not related to the study product if an alternative etiology can be documented and it is confirmed to be not related to the study product by the Independent Safety Monitor at the site.
[†] Subject at rest.

15.2.3 Additional Adverse Event Characterization and Severity Grading

Lesions at the ID challenge site will be characterized by the study site staff as follows:

1. Erythematous Macule: flat red spot.
2. Non-Erythematous Papule: a small, circumscribed, non-erythematous, palpable elevation in the skin.
3. Erythematous Papule: a small, circumscribed, erythematous, palpable elevation in the skin.
4. Vesicle: clear fluid filled papule.
5. Pustule: cloudy, purulent fluid filled papule.
6. Ulcer: an open lesion on the surface of the skin caused by superficial loss of tissue, often attended by the formation of pus.
7. Eschar/Crust: a lesion with an erythematous base and a central scab (a coagulation product of blood, serum, pus, or a combination of two or more of these).
8. Healed: previously observed lesion that has dried up without any persistent induration, ulcer, or eschar. Redness under an area of previous eschar formation seen after the eschar

has fallen off during the healing process would not be considered active erythema, but instead would be considered evidence of a healed lesion.

9. Scar: replacement of normal skin with fibrous tissue (other than keloid formation).
10. Keloid: nodular scar.

Severity Grading for Lymphadenopathy

The site principal investigator or appropriate sub-investigator licensed to make medical diagnoses will assess the severity of axillary, cervical, and epitrochlear lymphadenopathy bilaterally by applying the grading scale in the following table:

Table 5: Severity Grading for Lymphadenopathy

Grade	Definition
Grade 0 (none)	No palpable nodes, or all nodes < 1 cm (pea-sized), mobile, and nontender.
Grade 1 (mild)	At least one node ≥ 1 cm (pea-sized) but < 2.5 cm (cherry-sized), but mobile and non- tender or tender only with firm pressure.
Grade 2 (moderate)	At least one node ≥ 2.5 cm (cherry-sized) or tender to light touch or spontaneously reported as painful, but not causing significant limitation of normal everyday activities.
Grade 3 (severe)	At least one node that is tender to light touch or spontaneously reported as painful AND causing significant limitation of normal everyday activities. Any one of palpable fluctuance or heat, fixation to underlying tissues, or visible erythema.

Severity Grading for Clinical Safety Laboratory Adverse Events

Table 6: Severity Grading for Clinical Safety Laboratory Adverse Events - Hematology

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC (Increase) 10^3/UL	10.6 – 15.0	15.1 – 20.0	>20.0
WBC (Decrease) 10^3/UL	2.5 – 3.9	1.5 – 2.4	<1.5
HgB (Female) g/dL	10.1 – 11.4	8.5 – 10	<8.5
HgB (Male) g/dL	11.0 – 12.4	9.5 – 10.9	<9.5
Platelets (Decrease) 10^3/UL	125 – 139	100 – 124	<100

Table 7: Severity Grading for Clinical Safety Laboratory Adverse Events - Chemistries

Chemistries	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Creatinine mg/dL	1.31 – 1.70	1.71 – 2.00	>2.00
ALT (SGPT) (Female) IU/L	41 – 100	101 – 200	>200

ALT (SGPT) (Male) IU/L	56 – 138	139 – 275	>275

15.2.4 *Serious Adverse Events*

Serious Adverse Event (SAE):
An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death.
- A life-threatening adverse event*.
- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization, may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject 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.

  * 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 patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician.
- Recorded on the appropriate SAE form and eCRF.
- Followed through resolution or stabilization by a study clinician licensed to make medical diagnoses.
- Reviewed and evaluated by the SMC periodically. Related and unexpected SAEs will also be reviewed by the IRB.

15.2.5 *Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings*

The site principal investigator is responsible for reporting all AEs/SAEs that are observed or reported during the study, regardless of their relationship to study product. AEs/SAEs, abnormal laboratory test values or abnormal clinical findings will be documented, reported and followed appropriately. Adverse events will be assessed through approximately 8 weeks following Tice® BCG administration. Serious adverse events will be assessed for the study duration.

15.3 **Reporting Procedures**

- Document AEs from Visit 02 (Day 0) through Visit 10 (Day 56±2).
- Document SAEs from Visit 02 (Day 0) through Visit 15 (Day 114±1).

16.0 DATA AND SAFETY MONITORING PLAN

The Data Safety Monitoring Plan for this study includes the Safety Monitoring Committee (SMC) noted in Section 13.0 above and the safety assessments noted in Section 15.0. As this is an observational study using licensed products consistent with product labeling, external monitoring and a chartered committee with independent representation is not required.

The protocol will be reviewed at least annually and as needed by the Institutional Review Board (IRB). The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study. The study investigators will monitor for adverse events and will refer participants for local medical care if necessary.

17.0 DATA MANAGEMENT/CONFIDENTIALITY

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the Seattle MCTC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with all source documents or the discrepancies should be explained.

17.1 Data Management Responsibilities

All data collection forms must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

The Seattle MCTC will be responsible for data management, quality review, analysis, and reporting of the study data.

17.2 Data Capture Methods

Clinical data (including AE/SAEs, concomitant medications, physical assessments, and reactogenicity data) will be entered into the Seattle MCTC's Clinical Conductor® web-based 21 CFR Part 11-compliant Clinical Trial Management System. Clinical data will primarily be entered directly from the data collection forms.

17.3 **Types of Data**

Data for this study will include safety, laboratory (clinical and research), and outcome measures (e.g., reactogenicity and immunogenicity).

17.4 **Study Records Retention**

Records and documents pertaining to the conduct of this study, including eCRFs, data collection forms, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator indefinitely that is the policy of Fred Hutch.

The principal investigators will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The PI will also ensure conformity with ICH E6 Good Clinical Practice, and applicable federal regulations, guidance, and guidelines for Good Clinical Practice and Clinical Trials with humans.

17.5 **Quality Control**

The Principal Investigator will provide direct access to all protocol-related source data/source documents, and reports for the purpose of monitoring and auditing and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and current documentations are maintained on site. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training. The study staff will implement quality control procedures beginning with the data entry system; database quality control checks will be implemented. Any missing data or data anomalies will be updated for clarification and resolution.

18.0 **STATISTICAL CONSIDERATIONS**

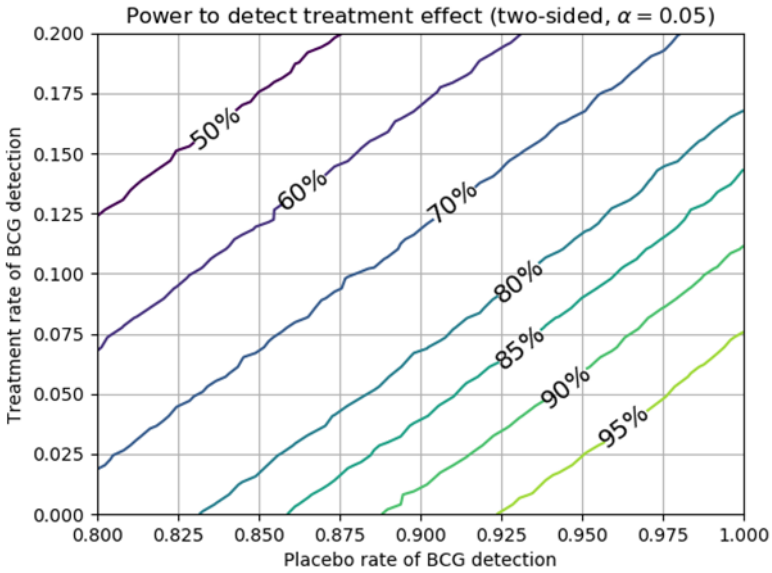
18.1 **Sample Size and Power for Outcome Measures**

The study is designed to demonstrate the safety, tolerability and feasibility of using intradermal BCG as a model for the evaluation of TB drugs. With 5 participants in each arm and a low number of AE/SAEs expected, the study is not powered for direct comparison of the two study groups. Instead, reactogenicity, AEs and SAEs will be presented with descriptive statistics summarizing each group. Interpretation of differences will rely heavily upon clinical judgment. In the rare event that there is a high frequency safety event of at least 25% in one of the groups there will be a 76% chance of observing at least one event and a 37% chance of observing 2 events in the relevant group. All participants are expected to provide safety data.

True event rate (%)	Pr(0/5)	Pr(≥1/5)	Pr(≥2/5)
5	0.77	0.23	0.02

10	0.59	0.41	0.08
15	0.44	0.56	0.16
20	0.33	0.67	0.26
25	0.24	0.76	0.37

It is expected that BCG will be detectable in all skin biopsies provided 4 days after BCG immunization and that the 3 day INH treatment will eliminate detectable BCG in all participants. Therefore, even with only 5 participants per group there will be power to detect a difference in the number of participants with detectable BCG either by qPCR or culture. The plot below shows that a low rate of BCG detection in the treatment arm (<0.05) and a high rate of detection in the control arm (>0.95) there will be >90% power to detect a difference between the groups (Fisher's exact test, two-sided α=0.05). Power will be diminished if the actual detection rates are shifted toward 0.5 for either group. It is assumed all participants will contribute data for this objective.



Descriptive statistical analyses will be conducted with the data generated from the Secondary Endpoints. The analyses will generate hypotheses to be tested in future studies.

18.2 **Randomization**

The participants assignment to either Group 1 and 2 will be computer generated.

18.3 **Ethnic and Gender Distribution Chart**

The target population reflects the Seattle King County community. Projected Target Accrual

ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	0	0	0

Not Hispanic or Latino	5	5	10
Ethnic Category Total of All Subjects*	5	5	10
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	4	4	8
More Than One Race	0	0	0
Racial Categories: Total of All Subjects*	5	5	10

19.0 ETHICS/PROTECTION OF HUMAN SUBJECTS

19.1 Institutional Review Board

The participating institution will provide for the review and approval of this protocol and the associated informed consent documents and recruitment material to the Fred Hutch IRB, registered with OHRP. Any amendments to the protocol or consent materials will also be IRB approved before they are placed into use.

19.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families. Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that their medical care will not be adversely affected if they decline to participate in this study.

19.3 Exclusion of Minors

Minors under the age of 18 are excluded from this study as there are no benefits for participation in the study.

19.4 **Subject Confidentiality**

Each subject will be assigned a unique subject number to protect subject confidentiality. Data collected for this protocol will be stored in a password-protected a database stored on secured servers or on coded paper documents in locked filing cabinets at the sponsor site. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number. Only trained study staff approved by the principal investigator will have access to subject data. In the even that a staff member leaves the center, subject data access will be revoked.

19.5 **Future Use of Stored Specimens and Data**

Data collected for this study will be analyzed and stored on secured, password-protected servers at sponsor site. After the study is completed, the coded data may be used by other researchers including those outside of the study. Permission for use of coded, deidentified data collected for the study for future research will be included in the informed consent.

With the participant's approval and as approved by Fred Hutch IRB, de-identified biological samples will be stored as part of a repository so it can later could be used to research the role of BCG in human challenge models and other inflammatory processes. The repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biological sample storage may not be possible after the study is completed.

20.0 **PUBLICATION POLICY**

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) 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.

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

21.0 **REFERENCES**

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22.0 **APPENDICES**

Appendix A: Lab Procedures Table

					Tube volume (mL)																
				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
				Day:	Screening visit	D0	D1	D2	D3	D4	D5	D6	D8	D15	D28	D56	D84	D98	D100	D112	D114	
				Week:		W0	W0	W0	W0	W0	W0	W0	W1	W2	W4	W8	W12	W14	W14	W16	W16	
						BCG	**—**	**—**	**Biopsy**	**INH**	**INH**	**INH**	**—**	**—**	**—**	**—**	**—**	**—**	**—**	**—**	**—**	
Procedure	**Ship to**		**Tube**	**Tube size (vol. capacity)**																		**Total**
BLOOD COLLECTION																						
Screening or diagnostic assays																						
IGRA	Local Lab	Local Lab	EDTA	5mL	4	—	—	—	—	—	—	—	—	—	—	—	—	4	—	4	—	12
Screening HIV test	Local Lab	Local Lab	EDTA	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
PPD	?	?	—	Injection	—	—	—	—	—	—	—	—	—	—	—	—	—	X	X	X	X	0
Safety labs																						
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
STI Serology																						
Syphilis	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Immunogenicity & Mycobacteria assays																						
HLA host genetics	CSR	FHCRC	ACD	8.5mL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
Cellular assays																		—	—	—	—	
ICS/ELISPOT / additional tests	CSR	FHCRC	ACD	8.5mL	—	51	—	—	—	—	—	—	51	—	51	51	51	—	—	—	51	306
Humoral assays																						
Ab Assays / additional tests	CSR	?	SST	8.5mL	—	17	—	—	—	—	—	—	17	—	17	17	17	—	—	—	17	102
Mycobacteria assays																						
BCG PCR	CSR	UW	—		—	—	—	—	—	X	—	—	X	X	X	—	—	—	—	—	—	0
BCG Invitro Culture	CSR	CIDR	—	Biopsy	—	—	—	—	—	X	—	—	X	X	X	—	—	—	—	—	—	0
Innate Immunity																						
Serum Cytokines	CSR	?	SST	3.5mL	—	3.5	3.5	3.5	3.5	3.5	—	—	3.5	3.5	3.5	3.5	3.5	—	—	—	—	35
Whole Blood Gene Expression	CSR	?	Tempus	3mL	—	3	3	3	3	3	—	—	3	3	3	3	3	—	—	—	—	30
STORAGE																						
PBMC	CSR	FHCRC	ACD	8.5mL	—	40	—	—	—	—	—	—	—	50	20	20	20	—	—	—	70	220
Visit total					**29**	**114.5**	**6.5**	**6.5**	**6.5**	**6.5**			**74.5**	**56.5**	**94.5**	**94.5**	**94.5**	**4**	**0**	**4**	**138**	**730**
56-Day total					**29**	**143.5**	**150**	**156.5**	**163**	**169.5**			**244**	**300.5**	**395**	**489.5**	**283.5**	**193**	**193**	**197**	**240.5**	
URINE COLLECTION																						
Urinalysis	Local lab	Local lab			X	—	—	—	—	—			—	—	—	—	—	—	—	—	—	—
Pregnancy test	Local lab	Local lab			X	—	—	—	—	—			—	—	—	—	—	—	—	—	—	—
Chlamydia/Gonorrhea	Local lab	Local lab			X	—	—	—	—	—			—	—	—	—	—	—	—	—	—	—

Appendix B: Participant Procedures Table

Clinic Visit	1 (Screening Visit)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Day	-42 to -1	0	1	2	3	4	5	6	8±1	15±1	28±1	56±2	84±3	98±1	100±1	112±1	114±1
Week	0								1	2	4	8	12	14		16	
Agents		BCG				INH	INH	INH									
Informed consent	X																
Medical history	X																
Height, weight: BMI	X																
Vitals (pulse, blood pressure)	X																
Urine collection	X																
Pregnancy test (blood)	X																
Pregnancy test (urine)	24 hr before to BCG dose																
General exam and Clinical assessment	X					X*			X*	X*	X*	X*	X*				X
Lymphadenopathy assessment						X*			X*	X*	X*	X*	X*				
BCG injection site evaluation		X	X	X	X	X	X	X	X	X	X	X	X				
Abnormal BCG injection site											*See section 11.3 for procedures*						
Abnormal BCG injection site clinic visits											*Clinic visits 2x/week from Day 28 ±1 until resolved*						
Abnormal BCG injection site photos											*Once/week from Days 28 ± 1 until resolved*						
Health status and AE/SAE review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant med review	X					X	X	X	X	X	X	X	X	X	X	X	X
Oral temperature reporting	X		Days 1 - 112 (recorded on Memory Aid eCRF device)														
AE/SAE reporting			Days 1 - 114 (recorded on Memory Aid eCRF device)														
Concomitant medication reporting			Days 1 - 114 (recorded on Memory Aid eCRF device)														
ID site pain, tenderness, drainage reporting			Days 1- 114 (recorded on Memory Aid eCRF device)														
Blood draw	X	X	X	X	X	X			X	X	X	X	X	X		X	X
Skin biopsy					O/S				X/S	O/S		X/S					
Photographs of ID site						X			X	X	X	X	X				X
PPD skin test														X	X	X	X
Dressing/guaze collection			X	X	X	X			X	X	X	X	X				

* Only if applicable due to medical history and clinical assessment

O = microbiology

X = immunology

S = swab

* Only if applicable due to medical history and clinical assessment
O = microbiology
X = immunology
S = swab