Preventing *Mycobacterium Tuberculosis* Infection in HIV-Exposed Infants

Short title: Infant TB Infection Prevention Study ("iTIPS")

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
BCG	Bacille Calmette Guerrin
BMCs	breast milk cells
CDC	Centers for Disease Control and Prevention
DNA	deoxyribonucleic acid
DAIDS	Division of AIDS
DSMB	Data Safety and Monitoring Board
EAE	expedited adverse event
EC	ethics committee
FDA	(United States) Food and Drug Administration
HEU	HIV-exposed uninfected
HIV	Human Immunodeficiency Virus
HLA	human leukocyte antigen
IGRA	interferon gamma release assays
IPT	isoniazid preventive therapy
INH	isoniazid
IRB	Institutional Review Board
KRTC	Kenya Research and Training Center
LTBI	latent tuberculosis infection
LFT	liver function test
MCH	maternal child health
MOH	Ministry of Health
MTB	*Mycobacterium tuberculosis*
PBMCs	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PMTCT	prevention of mother to child transmission
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
QFT-Plus	Quantiferon Plus
RCT	randomized controlled trial
SAE	serious adverse event
SUSAR	suspected unexpected serious adverse reaction
TLR	toll-like receptor
TB	tuberculosis
TST	tuberculin skin test
UW	University of Washington, Seattle

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SCHEMA

Purpose: To determine whether isoniazid (INH) reduces the risk of *Mycobacterium tuberculosis* (MTB) infection in HIV-exposed but uninfected (HEU) children. To determine the epidemiologic and immunologic correlates of MTB infection in HEU.

Design: Randomized controlled trial (RCT) of INH vs. no INH with nested sub-studies to evaluate epidemiologic and immunologic correlates of MTB infection, and to explore the role of INH in prevention of active TB and mortality.

Study Population: HIV-exposed uninfected (HEU) infants and their mothers

Study Size: 300 infants, 150 per arm

Treatment Regimen: Isoniazid (INH) ~10 mg/kg (7-15 mg/kg), will be administered once daily to infants in INH arm for 12 months.

Study Duration: 3 years (12 months of follow-up for each participant)

Primary Objectives:

- AIM: 1 Among HEU infants enrolled at approximately 6 weeks of age, compare the risk of acquiring MTB infection during 1 year of follow-up in infants randomized to receive INH vs. no INH using an IGRA assay to determine MTB infection status.

Secondary Objectives:

- AIM 2: Determine epidemiologic correlates of MTB infection among infants enrolled in the RCT.

- AIM 3: Determine immune correlates of risk of primary MTB infection and their potential interactions with INH. Assays will include infant peripheral blood BCG-specific T-cell responses at approximately 6 weeks post BCG vaccination, and maternal breast milk and peripheral blood MTB-specific T-cell responses at approximately 6 weeks postpartum.

Exploratory Objectives:

- Investigate the impact of IPT on a combined endpoint of MTB infection, TB disease, and death among HEU infants

Study Sites: Maternal Child Health (MCH) clinics in Western Kenya (Kisumu County Hospital, Jaramogi Oginga Odinga Teaching and Referral Hospital, Lumumba Sub-County Hospital, Ahero and Bondo).

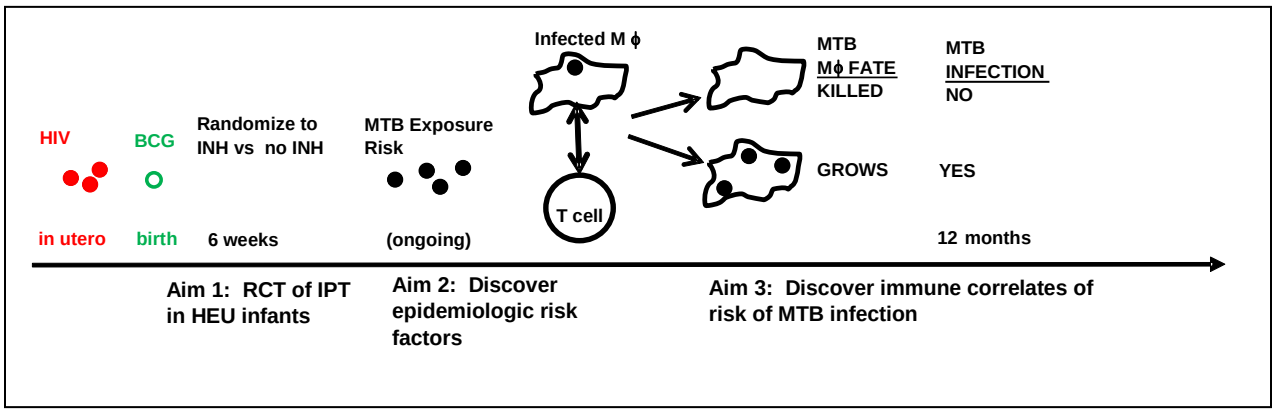

***Figure 1**: Aims of RCT to evaluate INH to prevent MTB infection in HEU infants*

OVERVIEW OF STUDY DESIGN AND RANDOMIZATION SCHEME

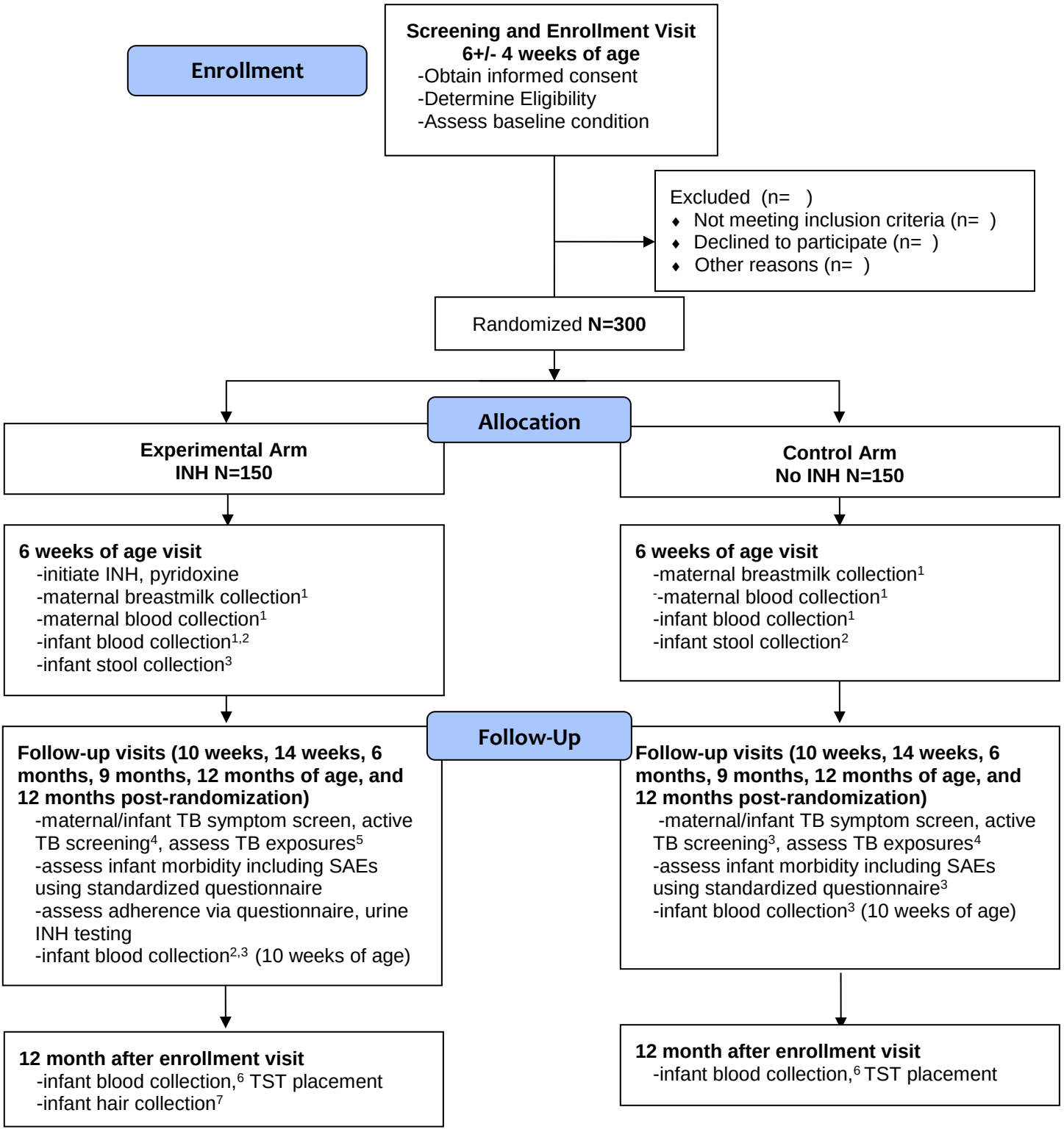

Figure 2: Study schema

[1]Aim 3: Immunologic correlates of MTB infection objective
[2]LFT in INH arm to monitor for SAEs
[3]Sample collection for future exploratory aims (infant gut microbiome, role of infant antibodies and infant MTB infection, role of IPT on BCG response)
[4]Exploratory aim (active TB)
[5]Aim 2: Epidemiologic correlates of MTB infection objective
[6]Aim 1: Blood drawn for IGRA to ascertain MTB infection status at 12 months
[7]for INH levels

1.0 INTRODUCTION

1.1 Background and Significance

Pediatric Tuberculosis (TB) Burden and Pathogenesis: Pediatric TB represents a major cause of childhood morbidity and mortality worldwide.[1,2] In a recent model from 22 high burden countries, it was estimated that in 2010 7.6 million children <15 years of age had *Mycobacterium tuberculosis* (MTB) infection, of whom over 650,000 developed active TB.[3] Children have different disease presentation than adults; with paucibacillary disease, rare development of cavitation, and more frequent miliary disease and TB meningitis.[4-7] Children have a higher rate of progression from infection to active disease than adults (50% <1 year, 20-30% at years 1-2, vs. 10-20% >10 years).[6,8] Pediatric TB disease occurs soon after primary exposure to MTB without pre-existing adaptive immune responses, and both innate and early adaptive immune responses may influence susceptibility.[4-7] Virtually all childhood TB disease reflects primary disease, whereas a significant portion of adult disease is due to reactivation of latent TB infection (LTBI).[2]

TB Risk and Outcomes in HIV-Infected and HIV-Exposed Uninfected (HEU) Children: In adults and children MTB infection is correlated with likelihood and intensity of exposure to an infectious TB case. Children living with HIV-infected household members are at increased risk of TB exposure.[9] Among children, accelerated progression from latent TB infection to active TB disease is associated with immunosuppression and younger age.[10] Kenya is one of 22 high TB burden countries with a generalized TB epidemic affecting young adults. A large observational study from AMPATH in Western Kenya longitudinally estimated TB disease incidence in HIV-infected children and noted a staggeringly high 17.1% annual incidence of active TB in a cohort with a median age of 1.0 year at enrollment.[11] TB disease prevalence was also high at 3.6% on

enrollment.[11] Other studies demonstrated similarly high rates of TB in HIV-infected children.[12-14] In a South African trial, which excluded infants with known household TB exposure, 12.6% of HIV-infected infants developed protocol-defined TB disease, and among HEU infants, 7.4% developed TB disease.[15] Despite exclusion of infants with known household TB exposure, risk of TB disease in HIV-infected and HEU infants is high, reflecting substantial community exposure to TB.

Isoniazid Preventive therapy (IPT) and TB: IPT has been used since the 1960s to treat latent tuberculosis infection and prevent progression to active TB disease. The famous Bethel, Alaska study was a household randomized trial with 6064 individuals randomized to placebo versus IPT. The NH recipients had a 55% reduction in active TB disease incidence with a benefit that persisted for >19 years.[16,17] Other studies have found similar levels of efficacy to prevent active TB.[18] Despite this demonstrated efficacy of INH to prevent active TB disease, a recent cluster-randomized trial of INH treatment for latent TB infection in adults in the South African gold mines did not demonstrate efficacy to prevent active TB.[19] Variable efficacy of IPT has also been observed in children. In a randomized trial of IPT in HIV-infected without reported TB exposure (N=548) and HEU (N=804) infants (enrolled at 91-120 days of life) in South Africa and Botswana, INH (given for 96 weeks) did not prevent TB disease in either group after 96-108 weeks of follow up.[20] Furthermore, in the HEU group, INH did not prevent MTB infection as measured by a single tuberculin skin test (TST) at week 96. In contrast, an RCT in South Africa randomized HIV-infected children >8 weeks (N=263) to INH vs. placebo (independent of reported TB exposure) and found that INH prevented TB disease and decreased mortality.[15] However, MTB *infection* was not assessed as an endpoint. **In summary, IPT is partially effective in adults and variably effective for preventing TB disease in HIV-infected and**

HEU children. The reasons for the partial and variable efficacy of IPT are not known. A recent study from Botswana indirectly suggests that IPT may prevent MTB infection among HIV-infected adults.[21] In this study, TST negative HIV-infected adults were demonstrated to receive benefit from IPT in prevention of active TB, suggesting a potential effect of IPT in preventing acquisition of MTB infection as well as prevention of progression to TB disease among those already with MTB infection. The effect of IPT on preventing MTB infection has only been addressed in only one pediatric study with use of a single TST as an endpoint.[20] The lack of baseline measurement of MTB infection in these studies means that MTB infection identified at study endpoint could represent either prevalent MTB infection acquired prior to IPT provision or incident infection occurring after IPT initiation. **Because interferon gamma release assays (IGRA) offer higher specificity and potentially higher sensitivity for detection of MTB infection in the presence of recent Bacille Calmette Guerrin (BCG) vaccine, it is plausible that using IGRAs as an endpoint would enhance ability to detect potential preventive effect on MTB infection.**

BCG Efficacy in HIV-Infected and HEU Children: BCG vaccine has been used in humans since 1921 and administered to >1 billion people globally, including millions of infants. Meta-analyses have noted benefits in preventing pediatric disseminated and meningeal TB disease.[22,23] However, estimates of BCG efficacy are highly variable and may be influenced by BCG strain, environmental mycobacteria, or host factors.[24] Until recently, it was believed that BCG did not prevent primary MTB infection. **Intriguingly, over the past decade, an emerging body of evidence suggests that, in fact, BCG may prevent primary MTB infection.** In several retrospective studies comparing TB-contacts with and without prior BCG, those with BCG were less likely to have latent TB infection as detected by IGRA. A recent meta-analysis demonstrated

an overall protective efficacy of 19% among 14 studies and 3855 participants.[25] New prospective studies of BCG immune responses and influence on MTB infection could reveal key BCG protective immune phenotypes. Incidence of MTB infection in HEU infants as measured by IGRA is ~10-20% during the first year of life, providing sufficient statistical power for immunologic studies comparing early BCG-specific T-cell responses in infants who do versus do not develop MTB infection.[26]

Impact of HIV Exposure on Mortality & Immune Response to BCG and MTB: In comparison to unexposed infants, HEU infants have higher overall mortality and an altered immune response to BCG vaccination and infection from several pathogens.[27-32] The altered immune response includes increased T-cell proliferation in response to BCG, but decreased polyfunctionality of T-cell responses to both BCG and *Bordetella pertussis.*[27] **Although these studies indicate that BCG-induced immune responses are altered in HEU infants, no studies have addressed whether BCG-induced immune responses are associated with a clinically relevant endpoint such as MTB infection or TB disease.**[33-39]

The Innate Immune and the Macrophage Response to MTB in HEU: From recognition to killing, the macrophage plays a central role in MTB pathogenesis.[40-54] The quality or function of early, non-specific innate immune responses in HEU children could be influenced by a hyper inflammatory intrauterine milieu and also affect the immune response to BCG vaccination. Few longitudinal studies have been performed that measure innate, macrophage, or adaptive immune responses in HEU before MTB infection.

Absence of an Efficacious TB Vaccine and New Strategies: Although vaccination with BCG offers some protection against childhood TB disease and possibly protection against adult MTB infection, its efficacy is not adequate for disease control and the correlates of protection are not known. Development of a more effective vaccine is a global priority and depends on a thorough understanding of the host response to MTB infection. Given rapid progress in the field of innate immunity, new generation vaccine adjuvants are becoming available to stimulate tailored immune responses.[55-57] To strategically inform TB preventive studies, molecular epidemiology studies are important to: 1) identify immunologic factors that increase risk of MTB infection; and 2) lead to innovative strategies for immune modulation through drugs or vaccines based on insights into the mechanisms of immune response identified.

1.2 Innovation

Our project has several innovative features that include:

HIV-TB Exposure and Co-Infection Research Infrastructure in Kenya with Longitudinal Cohorts: Our investigative team is uniquely poised to examine INH and MTB infection at Kenyan sites with experience in conducting epidemiologic, immunologic, and genetic studies for >25 years. Importantly, these studies included HEU infants with serial peripheral blood mononuclear cell (PBMC) banking and immunologic analyses, including maternal infant TB IGRA studies.

Examination of Pediatric MTB Infection in HEU Children: The population of HEU infants is growing as PMTCT programs succeed in preventing mother-to-child HIV transmission, and HEU infants have high risk of TB.

Immune Profiling: We propose to examine mechanisms of immunity to MTB with multi-parameter flow cytometry immunologic techniques.

Evaluation of BCG-Induced Immune Responses in HIV-Exposed Infants and Correlates of Risk of MTB Infection: By measuring immune responses to BCG vaccination after perinatal exposure to HIV, we have an opportunity to examine the immune response to a standardized in vivo stimulus AND determine whether these responses are associated with developing MTB infection. Previous studies have documented that HIV exposure alters infant immune responses to vaccination, but have not correlated these responses with an important longitudinal outcome such as MTB infection.

IPT & Prevention of MTB Infection: Currently, we do not know why IPT has variable and partial efficacy in children. A prospective birth HEU birth cohort can provide an efficient approach to probe this question.

Infant gut microbiome and risk of MTB infection and BCG response: We will also be collecting stool samples for cryopreservation for potential future studies evaluating the relationship between infant gut microbiome and risk of MTB infection and BCG response.

Infant antibodies and risk of MTB infection and BCG response: We will also be collecting infant PBMC and plasma samples for cryopreservation for potential future studies evaluating the relationship between titer and effector function of infant mycobacterial antibodies and to evaluate if early use of isoniazid preventive therapy modifies infant innate and adaptive immune responses to BCG.

INH metabolism and acetylation transferase 2 (NAT2) status: INH is primarily metabolized in the liver primarily through acetylation of NAT2, which is further converted by oxidation by cytochrome P450 2E1 to hepatotoxic metabolites. Genetic polymorphisms of NAT2 can associated with fast, slow, and intermediate phenotypes of acetylation. Fast acetylators can convert approximately 90% of INH to acetylisoniazid compared to 67% among slower acetylators. The relationship between acetylation status and INH hepatotoxicity is not clear. Initially it was assumed that rapid acetylators may have higher risk of hepatoxicity due to greater conversion of INH to hepatotoxic metabolites. However, in some series, slow acetylators have higher risk of hepatotoxicity. Acetylation status could potentially affect the efficacy of INH in terms of Mtb infection prevention. Faster clearance of INH could be associated with lower protection from Mtb infection. We will use already collected samples to ascertain NAT2 polymorphisms.

Hair analysis as an objective measure of INH exposure: Many drugs are incorporated from the systemic circulation into hair as it grows, and the concentration of medications in hair reflects drug uptake from the systemic circulation over weeks to months.[58] Our collaborators have developed methods to extract and analyze prevalent-use ARVs from hair, and demonstrated hair concentrations of ARVs are stronger predictors of treatment outcomes compared to self-reported aherance.[59-62] Only a small thatch of hair is required (approximately 30 strands), and rates of acceptability and feasibility of collecting hair samples for hair ARV monitoring in African and Asian settings have been high (>95%).[63,64] They have recently expanded their hair analysis expertise to assess INH concentration in hair,[65] including among children initiating TB treatment.[66] The assay has been validated over the linear dynamic range of 0.5–100 ng INH/mg of hair utilising 20–30 strands of human hair (~1–3 mg). Unlike phlebotomy, hair collection is noninvasive and does not require specific skills, sterile equipment, or specialized storage conditions.[60] The avoidance of phlebotomy in assessing drug adherence may be particularly desirable in pediatric populations.[64,66] Hair sample collection merely requires a pair of scissors and storage is at room temperature. Additionally segmental analysis of hair samples allows for

the assessment of adherence at various time points over the past few months since distance along the hair shaft serves as a marker of time.[67] Drug levels in hair can provide a more objective measure of adherance than self-report alone,[59,64,68] and information regarding adherance over longer time periods without the collection and storage issues associated with plasma, PBMCs, or dried blood spots.[69-72]

1.3 Supportive Preliminary Data

UW-Kenya Research Training Center (KRTC): Our UW-KRTC has successfully conducted collaborative HIV research in women and children for >25 years. This has included enrolment of >3,000 mother-infant pairs in longitudinal studies, numerous pediatric cohorts with detailed virologic and immunologic data, and studies of genetic markers including toll-like receptors (TLRs) and human leukocyte antigens (HLA) and their influence on HIV transmission and progression. Cohorts have had excellent retention (>90%), serial clinical evaluation by study pediatricians, and storage of PBMCs and DNA for molecular epidemiology studies. Studies from UW-KRTC have had translational impact in defining HIV transmission epidemiology and pathogenesis and have yielded >500 publications on HIV transmission or progression in high impact journals including *JAMA, Lancet, J Infectious Diseases, New England Journal of Medicine, AIDS,* and *Clinical Infectious Diseases*. Our team includes clinical researchers, immunologists, virologists, and molecular epidemiologists, with a focus on bench-to-bedside translational research.

Prospective Studies of TB IGRAs In HIV-Infected Mother-Infant Cohort In Kenya Using Repository: Among 333 HIV-infected pregnant women, we utilized cryopreserved PBMCs to conduct IGRAs and detected positive TB-specific IGRA responses in 42.7% of women. Women with positive IGRAs had significantly higher baseline median CD4 cell count (478 vs. 396 cells/mm^3, p=0.03). Positive T.SPOT.TB IGRAs were associated with increased likelihood of subsequent active TB (aOR 4.8 95%CI 1.2-19.7, p=0.03) and with infant TB or mortality.[73] Serial assays during pregnancy showed modest decline in magnitude of responses during pregnancy with stable responses postpartum.[74] **Among 6-month old infants born to HIV-infected mothers, we noted that 10.9% of 128 infants were IGRA positive.**[26] This suggests a cumulative incidence of TB infection of 20.6% among HIV-exposed infants.[26] These studies demonstrate high prevalence of latent TB among HIV-infected women in Kenya and high incidence of MTB infection and disease among infants born to HIV-infected women during the first year of life. <u>While infants had lower phytohaemagglutinin (PHA) responses, representing lower mitogen responses than mothers, MTB-specific responses were of comparable magnitude in infants and mothers.</u>

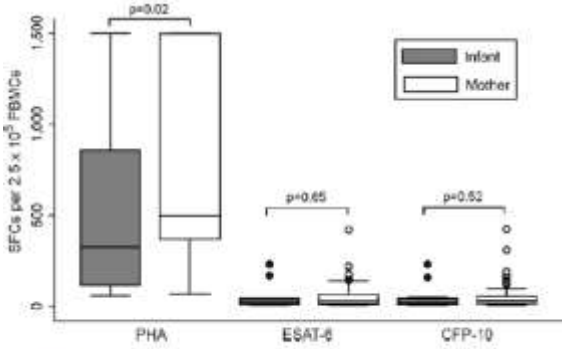

FIGURE 1: Magnitude of PHA, ESAT-6 and CFP-10 responses among mothers and infants with positive T-SPOT.TB.

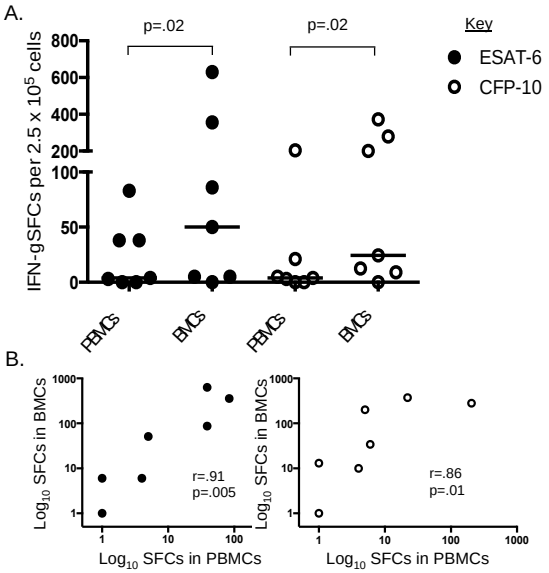

Figure 2: Magnitude and correlation of IFN-γ response to ESAT-6 and CFP-10 in Maternal BMCs and PBMCs. The T-SPOT.TB assay was performed on maternal PBMCs and BMCs. A. SFCs per 2.5 x 10^5 cells in response to antigens ESAT-6 (closed circles) and CFP-10 (open circles) are shown after subtraction of background in the nil control. Spearman's correlation of BMC and PBMC IFN-γ responses to ESAT-6 (closed circles) and CFP-10 (open circles) were assessed.

MTB-specific IFN-γ breast milk responses: We recently examined MTB-specific T-cell responses in

breast milk of HIV-infected mothers using the T-SPOT.TB IGRA.[75] HIV-infected women in Nairobi, Kenya were enrolled during pregnancy in 2002 and mother-infant pairs followed monthly for one year postpartum.[76] Breast milk and peripheral blood were collected at 1 month postpartum and breast milk cells (BMCs) and PBMCs were isolated and cryopreserved. Among 7 mothers with paired breast milk and blood assays, MTB-specific IFN-γ responses were higher in breast milk compared to blood (Fig. 2). The magnitude of IFN-γ responses in maternal breast milk and blood were correlated. Together, these data suggest that MTB-specific T-cell responses exist in BMCs. We will test whether these maternal responses are associated with protection from infant MTB infection in the current proposal.

Active TB Screening in HIV-Infected Mothers: Our study team, in collaboration with Drs. David Horne and John Kinuthia, has also established studies to screen mothers for active TB using maternal WHO symptom screening, culture, AFB microscopy, GeneXpert, and urine LAM (LaCourse, Cranmer, Horne, IJTLD Barcelona 2014, IDSA 2014). During a one-year period between July 2013 to July 2014, 306 HIV-infected women were enrolled at the Bondo and Ahero Maternal Child Health (MCH) sites, of which 288 had at least one adequate sputum culture. The median age was 26 years and 9% reported prior TB disease. Prevalence of culture-confirmed pulmonary TB was 2.4% (95% CI 0.98-4.9%) among the 288 women, irrespective of symptoms. Correlates of culture confirmed TB included cough >2 weeks (OR 8.9, 95% CI 1.6-51), household member with positive WHO TB symptom screen (OR 23, 95% CI 4.4-116), and TST >5 mm (OR 7.1, 95% CI 1.4-37). Overall, the sensitivity of symptom screen (43%) smear (0%), Xpert (43%), and LAM (0%) for pulmonary TB were low compared to culture. Among women with TST placed and who returned for reading, 12% were positive (95% CI 8-17%). Correlates of latent TB included age (OR 1.8 per 5 years, 95% CI 1.2-2.6), employment outside the home

(OR 2.6, 95% CI 1.1-6.3), and prior TB disease (OR 7.8, 95% CI 2.9-21). **This study illustrates the persistent burden of maternal and household TB to which HEU children are exposed.**

BCG-induced T-cell Responses in Infants Vaccinated with BCG: We and others previously discovered and characterized common TLR1, TLR5, and TLR6 non-synonymous coding region polymorphisms which regulate IL-6 secretion in monocytes after receptor stimulation.[46,77-82] These polymorphisms genetically define TLR1, TLR5, TLR6, and CD1A-deficient individuals.[83] These data illustrate that common innate immune deficiencies exist and can be used to examine the role of these genes in regulation of human innate and adaptive immune responses. We currently collaborate with Dr. Thomas Scriba at the South African TB Vaccine Initiative at the University of Cape Town (consultant on this grant) who is an expert on examining the immune response to BCG and MTB. Dr. Scriba and SATVI investigators (originally Drs. Hanekom, Hussey, Mahomed; currently Drs. Scriba and Mark Hatherill) established a study to discover BCG-induced immune correlates of risk for developing TB disease. A cohort of infants were vaccinated at birth with BCG (N=5650), had blood drawn at 10 weeks of age which was stimulated with BCG, and were followed for 2 years to determine who developed TB disease.[84,85] We examined whether genetic variation in the innate immune response was associated with BCG-induced T-cell immune responses. We discovered and published that individuals who are deficient in TLR1/6 signaling in myeloid cells have increased TH1-type T-cell responses after *in vivo* BCG vaccination.[86] To our knowledge, this was the first description of polymorphisms in innate pathway genes that affect the adaptive

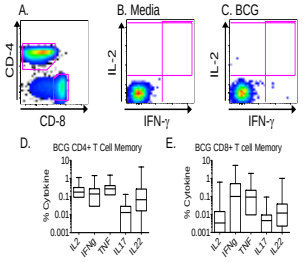

Figure 3: BCG-induced T-cell Responses in South African Infants. A whole blood cytokine assay was performed on 10 week old infants as described in the text. An 11 color flow cytometry panel with intracellular cytokine staining was performed with gating on CD4 and CD8 cells (A). Representative IL-2 & IFN-γ ICS plots depicted (B, C) along with frequencies of the CD4 and CD8-specific cytokines in 95 samples (D,E with median and IQR plotted).

response to *in vivo* vaccination against a bacterial pathogen in humans. We recently extended these studies to examine whether innate immune variation is associated with a broader repertoire of T-cell cytokine responses measured by intracellular cytokine staining. Using an 11-color flow panel (CD3, CD4, CD8, CD14, IFN-γ, IL-2, TNF, IL-4, IL-22, IL-17, CD154), we measured BCG-induced T-cell responses in blood samples obtained 10 weeks after vaccination. Frequencies of various BCG-specific cytokines are depicted in Figure 3. We are currently examining whether innate immune gene variants are associated with these T-cell responses. We are examining which genes regulate macrophage responses to MTB infection, by knocking down gene expression with siRNA, infecting macrophages with live MTB, and measuring binding, uptake, phagosome maturation, cytokine secretion by ELISA and replication.[46,77,78,81,86,87] Together, these data demonstrate that BCG-induced T-cell responses are detectable with a variety of techniques currently in use in our laboratory that will be used within the proposed project.

Summary of Preliminary Data: We have established a collaborative research site in Kenya that has been productive for >25 years with studies of HIV in women and children. We (GJS) broadened our research scope to include studies of TB over the past 5 years and have documented high rates of MTB infection in infants during the first year of life, MTB-specific IFN-γ breast milk responses, and potential TLR9 variants associated with MTB-specific T-cell responses. We (TRH) have also examined macrophage responses to MTB infection and the role of the innate immune response to BCG vaccination in other cohorts. Drs. John-Stewart (expertise in HIV epidemiology & clinical trials with women and children) and Hawn (expertise in innate immunity & immunogenetics of BCG vaccine responsiveness) have initiated collaborative studies to investigate the role of HIV and MTB during the immune response to BCG vaccination.

1.4 Rationale

HIV-exposed uninfected infants (HEU) in HIV/TB endemic settings have a high risk of MTB infection and TB disease, even in the absence of known MTB exposure. Because infancy is a time in which there is rapid progression from primary to active TB, it is important to define where, how, and when TB preventive interventions exert their effect and to build new strategies that adapt or extend approaches used in adults. Protecting HEU infants during this vulnerable, yet temporary, period of immunodeficiency may provide long term immunologic and mortality benefits. **The primary goal of this proposal is to determine whether INH prevents primary MTB infection in HEU infants. Additionally we will examine cofactors of primary MTB acquisition in the first year of life, and examine the role of immune protective mechanisms in this cohort.**

Among HIV-infected infants, 2 randomized control trials (RCTs) yielded conflicting data about whether IPT prevents TB disease and/or mortality. Only one of these evaluated HEU infants, and found no protective effect of IPT in decreasing TB disease. While previous IPT RCTs have focused on prevention of active TB disease, there are scant data regarding the impact of IPT on primary MTB infection. We recently found that, in 6-month old HEU infants, over 10% had evidence of MTB infection as detected by IGRAs, corresponding to a 20% annual cumulative incidence of infection. This suggests that HEU infants have a substantial incidence of MTB infection related to community and household TB exposure. There are no published prospective longitudinal studies of evaluating the role of INH to prevent MTB infection among HEUs using IGRA testing. Unlike TSTs, IGRAs can detect MTB infection and distinguish it from immune response to recent BCG vaccination. A prospective birth HEU cohort using IGRAs to detect MTB infection can provide an efficient approach to probe determinants of MTB infection, more

rapidly accruing endpoints (MTB infection) than studies of TB disease and this study design can contribute unique insights regarding mechanisms of prevention of primary MTB infection.

Current World Health Organization (WHO) guidelines recommend that all HIV-infected adult and adolescents living with HIV should be screened for TB with a clinical algorithm and those who do not report any one of the symptoms of current cough, fever, weight loss or night sweats are unlikely to have active TB and should be offered IPT.[88] Children with HIV >12 months of age with who are unlikely to have active TB based on symptom-based screening, and have had no contact with a TB case should receive six months of IPT (10 mg/kg/day) as part of a comprehensive package of HIV prevention and care services as well. However the current WHO recommendations for IPT do not recommend routine IPT for children <12 months of age with HIV due to the previously mentioned conflicting data in children < 12 months, and remain silent regarding the role of IPT in HIV-exposed but uninfected children. Given there is equipoise in whether INH prevents MTB infection, and whether it would prevent MTB infection specifically in among HEU children, a RCT design would provide important information regarding the efficacy of INH in preventing MTB infection in this population.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

- **AIM 1:** Among HEU infants enrolled at approximately 6 weeks of age, compare the risk of acquiring MTB infection during 1 year of follow-up in infants randomized to receive INH vs. no INH using an IGRA assay to determine MTB infection status.

2.2 Secondary Objectives

- **AIM 2:** Determine epidemiologic correlates of MTB infection among infants enrolled in the RCT.
- **AIM 3:** Determine immune correlates of risk of primary MTB infection and their potential interactions with INH. Assays will include infant peripheral blood BCG-specific T-cell responses at approximately 6 weeks post BCG vaccination, and maternal breast milk and peripheral blood MTB-specific T-cell responses at approximately 6 weeks postpartum.

2.3 Exploratory Objectives:

- Investigate the impact of INH on a combined endpoint of MTB infection, TB disease, and death among HEU infants.

2.4 Study Design

2.4.1 Participating Study Sites:

This study will be conducted in our collaborative maternal child health (MCH) research sites in western Kenya (Kisumu County Hospital, Jaramogi Oginga Odinga Teaching and Referral Hospital, Lumumba Sub-County Hospital, Ahero, and Bondo). We have enrolled pregnant HIV-infected and uninfected women and their infants in longitudinal studies at these sites for more than 4 years.

Figure 4: Overall Study Strategy

Study Design:	Non-blinded randomized control trial
Intervention:	Intervention: Infant INH for 12 months Control group: No INH
Primary Outcomes:	Aim 1: MTB infection in HEU infants at 12 months post enrollment as measured by IGRA (QFT-Plus) Aim 2: Epidemiologic correlates of infant MTB infection Aim 3: Immunologic correlates of infant MTB infection
Population:	HEU infants ~6 weeks of age and their HIV-infected mothers
Exclusions:	• Infants with known exposure to active TB in household • Positive HIV DNA at 6 weeks • Premature and/or < 2.5 kg
Target enrollment:	300 HEU infants and their HIV-infected mothers (150 each arm)
Sampling framework:	Consecutive enrollment of HEU infants and their HIV-infected mothers at MCH/PMTCT clinics, Nyanza region of Western Kenya

The study sites are embedded in the public sector routine MCH clinics. We have collaborated with CDC-Kenya Medical Research Institute (KEMRI) for TB microbiologic and IGRA studies at these sites for more than 4 years. HIV-infected mothers in Kenya are followed as part of the national PMTCT program and currently receive Option B+ triple antiretroviral therapy. Rates of mother-to-child HIV transmission at 6 weeks of age range from <1 to 10% in public MCH clinics that implement PMTCT screening and antiretroviral therapy administration. A recent estimate from Western Kenya of MTCT was 3% at 6 weeks of age. Our collaborative research team has conducted studies in Western Kenya sites for over 4 years and has extensive experience in recruitment of pregnant women and children into HIV research studies including RCTs with high rates of retention (Appendix V). Sites have defined TB referral clinics on the same campuses of the MCH clinics for women and children with suspected active TB.

2.4.2 Schedule of Study Visits and Procedures:

The study population will consist of HIV-exposed infants 6 weeks of age (within +/- 4 weeks), not premature and over 2.5 kg and their HIV-infected mothers. Infants with known exposure to household contacts with active TB at enrollment will be excluded from participation. In routine clinical care in Kenya, the majority of infants, including HEU infants, receive BCG vaccination (Tubervac-SII Russia strain, Serum Institute of India) at birth through the national immunization program. Documentation of BCG vaccination is provided on routine MCH immunization cards. Infants and mothers are then seen at routine postnatal visits, including at approximately 6 weeks postpartum. The proposed study will enroll and randomize infants to INH versus no INH at or close to the 6-week postpartum visit. Infants will be followed longitudinally for one year with clinical follow-up to assess for development of MTB infection. For the efficacy study and endpoint determination, 5 ml blood will be drawn at 12 months following enrollment to

determine infant MTB infection by an IGRA assay. QuantiFERON-TB Gold (QFT) is a specific IGRA which measures the amount of interferon-gamma (INF-γ) released by primarily CD4+ T helper lymphocytes after stimulation with TB-specific antigens (ESAT-6, CFP-10 and TB7.7) to measure MTB infection. We will also assess of the presence of MTB infection at the time of TB diagnosis of disease using QFT-Plus (same assay used to identify MTB infection status for the primary endpoint) and TST. Recently developed, QuantiFERON-TB Gold Plus (QFT-Plus) measures INF-γ released by CD8+ cytotoxic T lymphocytes as well, after stimulation with the same TB-specific antigens, which may have increased sensitivity in populations with lower CD4 counts including HIV.[89] In the event blood collected is insufficient blood volume (<4ml) for QFT-Plus, the blood collected be processed for a different test for M. tuberculosis infection. TST will also be placed when is blood is drawn as an additional measure of *M. tuberculosis* infection status. Infants will be screened at scheduled study visits that correspond with the Kenyan MOH visits (10 and 14 weeks of age, 6, 9, and 12 months of age) for any new known TB contacts, development of SAEs as well as symptoms concerning for active TB disease as part of our secondary and exploratory objectives. Liver function tests will be drawn at 6 and 10 weeks of age (at baseline and 1 month after INH initiation in the INH arm). For the immunologic studies, 5 mls of infant blood will be obtained at enrollment (approximately 6 weeks after BCG vaccination) and 1 month post-enrollment (10 week of age visit) as well as 30 mls of maternal breast milk and 5 ml of maternal blood on enrollment. Additionally we will collect stool samples from infants at enrollment. We will collect hair samples from children in the INH arm at the study endpoint visit 12 months post-randomization to measure INH exposure. We will measure infant immune responses in peripheral blood and maternal immune responses in peripheral blood and breast milk as outlined below. Our primary analytic goal of these immunologic exploratory

analyses is to determine which of these responses are associated with acquisition of MTB infection during 1 year of follow-up.

EXTENDED FOLLOW-UP AT 24 MONTHS OF AGE: AFTER COMPLETION OF THE PRIMARY TRIAL, PARTICIPANTS WILL BE INVITED TO RETURN FOR AN EXTENDED FOLLOW-UP VISIT AT 24 MONTHS OF AGE FOR A BLOOD DRAW AND TST PLACEMENT. 5-10 MLS OF BLOOD WILL BE OBTAINED AND WILL BE USED TO DETERMINE MTB INFECTION STATUS AND MEASURE INFANT IMMUNE RESPONSES. STANDARDIZED QUESTIONNAIRES USED DURING THE PARENT TRIALS WILL BE USED TO EVALUATE INTERIM INFANT AND MATERNAL HEALTH AT THE EXTENDED FOLLOW-UP VISIT.

3.0 STUDY POPULATION

HIV-exposed uninfected (HEU) infants and their HIV-infected mothers will be included in this study. Participants will be selected for the study according to the criteria in Section 3.1 and 3.2 [and the guidelines in Section 3.4]. They will be recruited, screened, and enrolled as described in Section 3.3 [and assigned to the intervention or control group as described in Section 7.4]. Issues related to participant retention and withdrawal from the study are described in Sections 3.5 and 3.6, respectively.

3.1 Inclusion Criteria

HEU infants who meet all of the following criteria are eligible for inclusion in this study:

- Aged 6 weeks within (+/- 4 weeks)
- Born to HIV-infected mothers
- Not premature and over 2.5 kg

3.2 Exclusion Criteria

HEU infants who meet any of the following criteria will be excluded from this study:

- Infants with known exposure to active TB in household
- Premature and < 2.5 kg

3.3 Recruitment Process

Recruitment: HIV-infected mothers with HEU infants will be informed about the study starting from 2 weeks postpartum and will be invited to enroll their infant in the study. We will recruit eligible HIV-infected mothers and their infants from MCH/PMTCT sites between 2-10 weeks after birth and enroll and randomize HEU children to INH vs. no INH at 6 (+/- 4) weeks of age. We anticipate the majority of infants will be recruited during their routine 6 week immunization visits, but have allowed an additional 4-week window for infants presenting early/late for this visit. HIV-exposed infants are routinely tested for HIV at ~6 weeks of age, however results of that test may not be available for a few weeks.

Study staff will work in conjunction with antenatal and pediatric staff at the MCH clinics to aid in our ability to identify potential participants. Interested mothers of infants will have the study explained to them, will have their questions answered, and will be asked to provide written consent (or thumbprint in the case of illiteracy). We have successfully recruited mother-infant pairs at these MCH/PMTCT sites for >4 years.

Study staff will help any women or infants with suspected tuberculosis to access care at TB clinics, as well as HIV care clinics if necessary. This insures that care for all women and children in this maternal and child health setting is not compromised by the presence of the study, and should ensure that subjects do not feel pressure to participate in the study to receive any postnatal, pediatric, TB or HIV-related services. Study staff have been working side by side with clinic staff at these sites for the last 4 years. Study staff are well trained in recruitment and are knowledgeable in recruitment without persuasion/coercion.

Enrollment: On enrollment, a study nurse will administer a standardized questionnaire that addresses sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and history, and ascertains current maternal TB symptoms (using WHO symptom screen) and

household symptoms. Mothers with suspected TB by WHO screen will be referred to the TB program for sputum TB screening and if found to have active TB, will be ineligible for participation and infants will receive INH for known active TB exposure per current Kenya national guidelines. Mothers will undergo physical examination with weight, height and BMI estimation; medical records will be used to abstract data on ART regimen, other medications, maternal HIV viral load and CD4 cell counts. Infants will be undergo physical examination and medical records and MCH cards will be used to abstract maternal prior PMTCT ART, infant PMTCT prophylaxis, birth weight, BCG vaccination date, and intercurrent illnesses and vaccines. At enrollment, infants will be examined and growth measures (weight, length, head circumference), mid-upper arm circumference, and presence of BCG scar will be determined. A questionnaire will address infant feeding and symptoms (including cough and fever). Infant blood will be collected for baseline PBMC separation and IGRA assay. Maternal breastmilk and maternal peripheral blood will be collected. Additionally infant stool will be collected for cryopreservation for future gut microbiome studies. At enrollment, among mothers who consent, household locator information, HIV care medical identification number, and cell-phone contacts will be obtained to facilitate tracing.

Randomization: Block site-stratified randomization will be used to allocate infants 1:1 to INH or no INH trial arms. Randomization numbers will be generated at UW prior to study start (under leadership of Dr. Richardson and with the UW CFAR Biostatistical Core).

3.4 Co-Enrollment Guidelines

Infants should not be enrolled in other TB prevention or TB vaccine studies because they might affect ascertainment of primary and secondary endpoints. For example an infant enrolled in a vaccine or other TB prevention trial may affect that infant's risk of MTB infection irrespective of INH or no INH administration.

3.5 Participant Retention

Once a participant enrolls in this study, the study site will make every effort to retain him/her for 12 months of follow-up in order to minimize possible bias associated with loss-to-follow-up. Participant retention procedures will be established such that loss rates do not exceed the incidence rate of the primary study outcome. Study site staff are responsible for developing and implementing local standard operating procedures to target this goal. Components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.
- Thorough explanation of the importance of the study treatment group to the overall success of the study.
- Collection of detailed locator information at the study Enrollment Visit, and active review and updating of this information at each subsequent visit.
- Use of appropriate and timely visit reminder mechanisms including cell phone SMS.
- Follow-up on missed visits.
- Mobilization of trained outreach workers or "tracers" to complete in-person contact with participants at their homes and/or other community locations.
- Mothers of infants who miss their monthly study visit will be contacted by phone or home visit and encouraged to continue follow-up, particularly for the 12 month IGRA visit (primary endpoint).
- Caregivers will be counseled on importance of INH adherence and adherence will be assessed using pill counts at monthly re-fill visits.
- Study visits are aligned with routine medical care (child immunization and maternal ART visits). We anticipate following mother-infant pairs at visits aligned with routine immunization, pediatric, and maternal ART visits.
- Travel reimbursement.
- Participants who discontinue treatment shall be maintained in follow-up as originally scheduled whenever possible.

- Extended follow-up at 24 months: for those participants who may have exited the study prior to consenting for a 24 month visit, participants will be re-contacted and given the option to consent for another follow-up visit at 24 months of age.

3.6 Participant Withdrawal

Regardless of the retention methods, participants may voluntarily withdraw from the study for any reason at any time. Participants also may be withdrawn if the study sponsor, government or regulatory authorities, or site IRBs/ECs terminate the study prior to its planned end date.

Every reasonable effort will be made to complete a final evaluation (Appendix I) of participants who terminate prior to the final study visit, including measuring *M. tuberculosis* infection status at the time of study exit. Study staff will record the reason(s) for all withdrawals from the study in participants' study records.

4.0 STUDY TREATMENT

4.1 Treatment Content

Isoniazid ~10 mg/kg (7-15 mg/kg) will be administered once daily to infants in the INH arm for 12 months. WHO dosage for INH is 7-15 mg/kg and CDC recommends 10-15 mg/kg; the South Africa/Botswana RCT used 10-20 mg/kg dosing. The Kenya Ministry of Health (MOH) recommends ~10 mg/kg and has standardized weight-based dosing (by weight band using 100 mg scored tablets) which correspond to WHO dosing recommendations[90,91] (APPENDIX II) and will provide INH for the study. Infants assigned to the control arm will not receive INH. Current Kenyan guidelines recommend IPT (isoniazid preventive therapy) for all TB-exposed children <5 years of age and for all HIV-infected children >1 year of age. The guidelines illustrate the uncertainty regarding IPT for <1 year olds with HIV infection, following the RCT from South Africa/Botswana that failed to demonstrate IPT effectiveness in <1 year olds. However, we

speculate that among HEU children exposed to community TB or unperceived household TB, INH may prevent MTB infection as detected by IGRA. Although data are conflicting, some adult studies noting benefit of longer periods of IPT (36 months versus 6 months) and demonstrating IPT benefit in TST negative HIV-infected adults suggest that IPT may confer protection from primary MTB infection. Pyridoxine will be provided children to decrease the risk of INH-associated peripheral neuropathy in the INH arm using Kenyan MOH weight-based dosing (5-7 kg ¼ 50 mg tab, 8-14 kg ½ 50 mg tab) (APPENDIX III) and will be provided by the MOH.

4.2 Treatment Administration

Participants in the experimental arm will be given their daily INH and pyridoxine by caregivers for 12 months. Caregivers of infants in the experimental arm will be given 1 month supplies of INH and pyroxidine at monthly med pick up visits.

4.3 Treatment Supply and Accountability

The Kenya Ministry of Health will provide INH and pyroxidine for the RCT. Study staff will maintain complete records of all study drugs received and subsequently dispensed to study participants. All unused meds will be returned to the Kenyan MOH after the study is completed or terminated.

4.4 Adherence Assessment

Caregivers will be counseled on importance of INH adherence and adherence will be assessed using pill counts at monthly re-fill visits. In addition, we will assess isoniazid in urine at follow-up visits using in-house urine test strips which are inexpensive (1.5 cents per strip) and have high sensitivity and specificity for detection of isoniazid in African adult and pediatric

populations.[92,93] We will also collect hair at 12 months post-enrollment at the study endpoint to assess for isoniazid levels as a more objective measure of adherence over time.

4.5 Toxicity Management

INH is well tolerated in pediatric populations. INH is metabolized in the liver and excreted primarily through the kidneys. Hepatotoxic effects are rare in children but can be life threatening. In children given recommended doses, peripheral neuritis or seizures caused by inhibition of pyridoxine metabolism are rare, and most do not need pyridoxine supplements. Pyridoxine supplementation is recommended for exclusively breastfed infants and for children and adolescents on meat- and milk-deficient diets; children with nutritional deficiencies, including all symptomatic HIV-infected children; and pregnant adolescents and women. In this study all infants in the INH arm will be provided with pyridoxine. For infants and young children, isoniazid tablets can be pulverized. IPT has been safe in prior RCTs and is administered routinely to TB-exposed infants. Routine liver function monitoring is not recommended during INH in children, however baseline liver function tests will be drawn at enrollment (6 weeks of age) and at 10 weeks of age (1 month after INH initiation) in those infants randomized to INH.

If toxicity is suspected, study administered drug will be immediately discontinued and in the case of concern for hepatoxicity, liver function tests (LFTs) will be performed. For this study, we will use the NIH Division of AIDS (DAIDS) Table for Grading the Severity of Pediatric Adverse Events to screen for eligibility and to grade clinical and laboratory toxicities and can be found at http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf. If there is an increase of LFTs to > Grade 3, we will follow the study participant until resolution of the toxicity to ≤ Grade 2. For > Grade 3 hepatic abnormality supported by repeat

laboratory tests: we will hold INH for two weeks and recheck LFTs in 2 weeks. If a > Grade 3 abnormality is still present after withholding INH for two weeks: continue to hold INH and recheck LFTs in 2 weeks.

4.6 HIV Seroconversion

HIV-exposed infants in Kenya are routinely tested at ~6 weeks of age using HIV DNA PCR. On enrollment, we may not yet have the results of the 6 week testing. Infants found to be HIV-infected from their 6 weeks of age testing will be followed throughout the study period, but will be excluded from the primary analysis. A recent estimate from Western Kenya of MTCT was 3% at 6 weeks of age.

Infant HIV status will be determined at exit using repeat HIV PCR DNA testing at KEMRI/CDC laboratories to confirm that infants remain HIV uninfected. Infants found to be HIV-infected at study exit will also be excluded from the primary analysis. All infants found to be HIV-infected will be immediately referred to the on-site pediatric HIV care clinics.

4.7 Active TB screening and diagnosis, TB exposure in infants

At scheduled follow-up visits intercurrent infant morbidity will be evaluated using standardized questionnaires. Both mothers and infants will be evaluated with standard TB screening questions regarding their own and household TB exposures. Any mother or infant with suspected active TB will be referred for TB microbiologic testing and X-rays, and these results will be abstracted to the Study database. Mothers with suspected active TB will be offered sputum AFB and GeneXpert testing consistent with Kenyan Ministry of Health guidelines. Infants with suspected TB will have chest X-ray, gastric aspirate testing by GeneXpert, and clinical review and classification as definite, probable or possible TB using Graham and NIH/WHO 2014 criteria.[94] Infants with report of close contact with TB case will be undergo evaluation for active TB. If active TB is ruled out, infants who are in the control (no

INH arm) will be referred to the MOH TB programme clinic for IPT evaluation per Kenyan guidelines. We will also assess of the presence of MTB infection at the time of TB diagnosis of disease.

4.8 Concomitant Medications

Enrolled study participants may continue use of all concomitant medications — except those listed under criteria for exclusion or treatment discontinuation — during this study.

All concomitant medications [taken or received by participants within the 2 weeks prior to study enrollment] will be reported on applicable study case report forms. In addition to prescribed and over-the-counter medications, other traditional preparations will be recorded. Medications used for the treatment of AEs that occur during study participation also will be recorded on applicable study case report forms.

5.0 STUDY PROCEDURES

An overview of the study visit and procedures schedule is presented in Appendix I.

6.0 SAFETY MONITORING AND ADVERSE EVENT

6.1 Safety Monitoring

Close cooperation between the Protocol Chairs, Investigators, study biostatistician, DSMB, and other study team members will be necessary in order to monitor participant safety and respond to occurrences of toxicity in a timely manner. Before the study begins, the team will decide on a schedule to hold regular conference calls during the period of study implementation, and additional ad hoc calls will be convened if required.

The Protocol Chairs/Investigators are responsible for continuous close monitoring of all adverse events (AEs) that occur among study participants, and for alerting the rest of the protocol team if unexpected concerns arise. A decision to stop the trial may be made by the protocol team at this

time, or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

6.2 Adverse Event Definitions and Reporting Requirements

6.2.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. The most frequent adverse events observed with INH are peripheral neuropathy and hepatotoxicity. For hepatotoxicity we will use the DAIDS Table for the Grading Severity of Pediatric Adverse Experiences (also referred to as the "Toxicity Table") which is available on the RSC website: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf. For peripheral neuropathy, we will use the tables in Appendix IV regarding the measurement of peripheral neuropathy by grade as well as age appropriate measures of peripheral neuropathy.

Study participants will be provided a 24-hour telephone number and instructed to contact the study clinician to report any AEs they may experience, except for life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be paged or otherwise contacted upon their arrival. With appropriate permission of the participant, whenever possible records from all non-study medical

providers related to AEs will be obtained and required data elements will be recorded on study case report forms. All participants reporting an AE will be followed clinically, until the AE resolves (returns to baseline) or stabilizes.

Study site staff will document on study case report forms all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. All AEs will be graded using the DAIDS Table for the Grading Severity of Pediatric Adverse Experiences (also referred to as the "Toxicity Table") which is available on the RSC website: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf. The investigator or designee will assess the relationship of all AEs to the study product based on the Investigator's Brochure, Package Insert, DAIDS Drug Risk List, and his/her clinical judgment. These documents are all available at http://rsc.tech-res.com/Document/safetyandpharmacovigilance/.

6.2.2 Serious Adverse Event

For the purposes of this study, serious adverse event (SAE) will be defined as an AE occurring that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization or prolongation of existing hospitalization

This includes important medical events that may not be immediately life-threatening or result in death, or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

Hospitalization itself is not an adverse event, but is an outcome of the event. The following types of hospitalization as related to our study do not require expedited reporting:

- Any admission unrelated to an AE (e.g. for administrative, or social admission for temporary placement for lack of place to sleep)
- Admission for diagnosis or therapy of a condition that existed before receipt of study agent(s) and has not increased in severity or frequency as judged by the clinical investigator

6.2.3 Adverse Event Reporting

6.2.3.1 Adverse Event Reporting to DSMB

For the purposes of this study we will use the definitions of Adverse Events (AEs) are outlined in DAIDS EAE Manual, which is available on the NIH RSC website at http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf. Although the study is not sponsored by NIH, we will use their recommendations on reporting and grading adverse events. Adverse events will be reported to DSMB.

6.2.3.2 Reporting Requirements for this Study

In addition to the EAE Reporting Category identified above, other AEs that must be reported in an expedited manner are: hepatic failure, peripheral neuropathy, diagnosis of active TB, diagnosis of HIV.

6.2.3.3 Grading Severity of Events

The most current Division of AIDS Table for Grading the Severity of Pediatric Adverse Events (DAIDS AE Grading Table) is used and is available on the RSC website at http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf .

6.2.3.4 Expedited AE Reporting Period

The expedited AE reporting period for this study is 6 month post study completion. After the protocol-defined AE reporting period, unless otherwise noted, only suspected unexpected serious adverse reaction SUSARs as defined in the EAE Manual will be reported to the DSMB if the study staff become aware of the events on a passive basis (from publicly available information).

7.0 STATISTICAL CONSIDERATIONS

7.1 Review of Study Design

Using a non-blinded randomized control study design, we will compare the risk of acquiring MTB infection among HEU infants enrolled at approximately 6 weeks of age during 1 year of follow-up between infants receiving INH vs. no INH using an IGRA assay to determine MTB infection status.

7.2 Endpoints

7.2.1 Primary Endpoints

Consistent with the primary study objective to compare the incidence of primary MTB infection in HEU infants randomized to receive INH vs. no INH, the following endpoint will be assessed:

- MTB infection as detected by interferon gamma release assays (IGRA) at 12 months post-enrollment.

7.2.2 Secondary Endpoints

Consistent with the secondary study objective to investigate epidemiologic correlates of MTB infection **(AIM 2)**, the following correlates will be assessed:

- Maternal HIV viral load and CD4 cell counts, maternal prior PMTCT/ART regimens, infant PMTCT prophylaxis
- Birth weight, BCG vaccination status and timing, and intercurrent illnesses and vaccines
- Growth measures (weight, length, head circumference, mid-upper arm circumference)
- Infant feeding and symptoms (including cough, fever, weight loss, or growth faultering)
- Infant HIV status at study enrollment and study end
- Report of known TB exposure, maternal IPT status

Consistent with the exploratory study objective to determine the immunologic correlates of MTB infection **(AIM 3)**, the following correlates will be assessed:

- Infant peripheral blood BCG-specific T-cell responses at approximately 6 weeks post BCG vaccination
- Maternal breast milk and peripheral blood MTB-specific T-cell responses at approximately 6 weeks postpartum.

7.2.3 Exploratory Endpoints

Consistent with the exploratory study objective to investigate the impact of INH on a combined endpoint of MTB infection, TB disease, and death the following events will be assessed:

- MTB infection as measured by IGRA at 12 months post-enrollment
- TB disease including microbiologically confirmed (culture or Xpert positive), or probable TB (clinical diagnosis).
- Death of HEU infant

7.3 Accrual, Follow-up, and Sample Size

Sample Size Estimations Aim 1: Number needed per arm to detect a maximum HR benefit of INH to decrease MTB infection as measured by IGRA positive at 12 month follow-up. With 125 infants in each arm we would have power to detect at least a 65% decrease in MTB infection in INH arm vs. control if cumulative incidence of positive IGRA in the control arm at 12 months follow-up is 0.2, or to detect a 70-80% or higher (HR 0.3-0.2) decrease if the cumulative incidence of positive IGRA in control arm is 0.15 or 0.1 (Table 1). **We will increase sample size by 20% to account for loss to follow-up, non-adherence, and isoniazid resistance, enrolling 300 mother-infant pairs (150 per arm).**

Table 1: AIM 1 Sample Size Estimates (Gray shaded close to study target)

Power 80%, 2-sided p 0.05 1 year follow-up	Maximum HR for IPT detectable	Risk of positive IGRA at m12	Number per arm
0.2 risk IGRA positive after 12 mos follow-up	0.5	0.2	220
	0.4	0.2	150
	0.35	0.2	120
	0.32	0.2	100
	0.2	0.2	55
0.15 risk IGRA positive after 12 months follow-up	0.5	0.15	300
	0.4	0.15	180
	0.35	0.15	150
	0.31	0.15	120
	0.2	0.15	75
0.10 risk IGRA positive after 12 months follow-up	0.5	0.1	420
	0.4	0.1	270
	0.35	0.1	220
	0.3	0.1	180
	0.2	0.1	110

We have estimated a substantive INH effect (65% decrease), which is consistent with IPT literature for active TB but completely undefined for MTB infection risk and one that may be persuasive for implementation. A larger sample size may be useful if prevalence of IGRA positivity is lower than we anticipate or if INH is less effective in prevention of MTB infection.

7.4 Random Assignment / Study Arm Assignment

Site-stratified randomization will be used to allocate infants 1:1 to INH or no INH trial arms. We will use block randomization with block size of 4. Randomization numbers will be generated at

UW prior to study start (under leadership of the Study Biostatistician Barbra Richardson and with CFAR Biostatistical Core).

7.5 Blinding

We have designed the study to be a non-blinded RCT to enable prompt clinical management of children in each arm, given an understanding of drugs that they are receiving. With non-blinded trials there are concerns about differential reporting and clinical management; however, our endpoint (IGRA status) will be assessed in the KEMRI CDC laboratory, which will be blinded to INH status of the participant. This endpoint is robust and not influenced by unblinded trial design.

7.6 Data Analysis

7.6.1 Primary Analyses

The primary analysis will be comparison of proportion IGRA positive at 12 months following enrollment between INH and no INH arms among HEU infants. Baseline maternal and infant characteristics, including maternal CD4, ART regimen, age, TB exposure, reported TB household symptom screen, maternal TB history prior to enrollment, employment and infant birth weight, gestational age, and weight and height z-scores (WAZ, HAZ) and MUAC at enrollment, will be compared between randomization arms to assess adequacy of randomization. The primary analysis will be a comparison of proportion of infants in INH vs. control arm with IGRA positive assays using Chi square tests using an intent-to-treat analysis. Infants who are found to be HIV DNA positive at enrollment or at study end will excluded from this ITT analysis of our primary endpoint. We do not anticipate baseline immune responses to TB antigens at enrollment at ~6 weeks of age. However, if we find baseline responses, we will conduct additional modified intent-to-treat analyses, incorporating data from baseline assays (that utilize

cryopreserved samples) to exclude any infant with evidence of immune responses to ESAT-6 or CFP-10 at enrollment visit. Thus, the modified intent-to-treat analysis will compare incidence of new MTB infection subsequent to randomization in INH versus no INH trial arms among HEU infants. All missing data will be assumed as missing completely at random.

7.6.2 Secondary Analyses

AIM 2: Exposures to be evaluated as potential correlates of MTB infection: There are scant data on determinants of MTB infection in HEU infants. A combination of household and community TB exposures may contribute to infant risk. The RCT offers an opportunity to explore contributors to MTB acquisition in nested case-control studies overall and in the control arm alone. Variables to be considered include those associated with increased likelihood of infant *exposure to active TB* and those that reflect infant host susceptibility factors. In terms of potential TB exposure, mothers with low CD4 count, paternal positive HIV status, maternal employment, crowding, and household TB symptoms may be associated with likelihood of TB exposure.

Potential *infant determinants* of MTB susceptibility include infant growth (WAZ, HAZ, WHZ) with indication of underweight (WAZ <-2) and stunting (HAZ<-2) at enrollment at 6 weeks or over the course of follow-up. Alternatively, growth deficits may accompany nutritional deficits, which could compromise immune responses to TB antigens and fail to reveal a positive assay despite MTB infection.

Table 2. Aim 2 Sample size estimates (Gray shaded adequate power)

Case-control (1:3, 34 cases: 102 controls) Alpha 0.05	Power if prevalence of exposure in controls is:		
	20%	**40%**	**50%**
OR 2	36%	41%	39%
OR 3	73%	78%	74%
OR 4	91%	93%	90%

Sample size considerations: For the nested studies to determine correlates of incident MTB infection, we

anticipate 34 cases with all other infants without evidence of MTB infection as potential controls. In sample size estimates assuming at least 3 controls in comparison group, there is between 73% to >90% power to determine ORs of 3 to 4 if prevalence of exposure in controls is between 20 to 50% as in Table 2 above.

AIM 2 Analyses: To identify epidemiologic correlates of MTB infection among infants enrolled in the study, cryopreserved specimens from the baseline visit will be assessed for responses to TB antigens concurrent with other assays outlined in immune correlates in the next paragraph. Any infant with positive IGRA at baseline will be excluded from cofactors analyses. We anticipate 25 MTB infections in the control arm and ~9 in the INH arm (assuming INH is 65% effective). In nested case-control analyses, infants who had IGRA conversion suggestive of new primary MTB infection will be compared to those with valid negative assays at 12 months. Univariate and multivariate logistic regression will be used to compare mother-infant characteristics of infants with and without IGRA positive assays at month 12. We will initially build nested case-control studies incorporating all MTB infections from both arms of the RCT and then conduct stratified analyses in each trial arm to evaluate potential cofactors that are modified by INH.

Aim 3. Determine immune correlates of risk of primary MTB infection and their potential interactions with INH. Assays will include infant peripheral blood BCG-specific T-cell responses 6

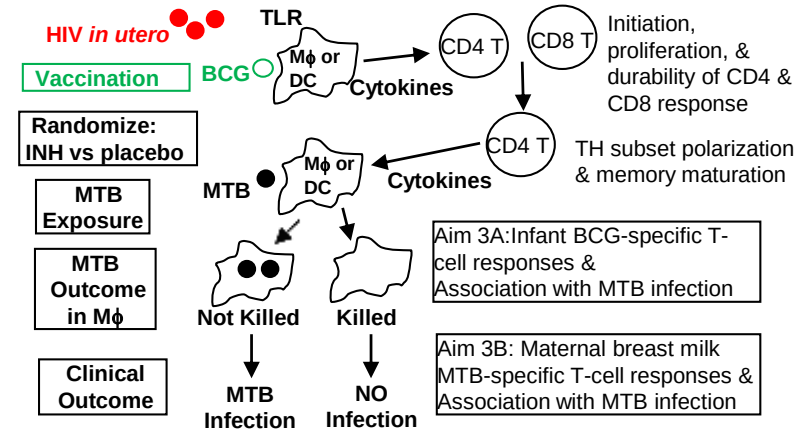

Fig. 5: Model of HIV exposure & effects on adaptive immune responses to BCG and risk of MTB infection: BCG vaccination in HEU infants activates macrophages and dendritic cells which prime T cells and the adaptive immune response to activate macrophages to kill MTB and orchestrate clinical outcomes. DC, dendritic cell; Mϕ, macrophage.

weeks post-vaccination and maternal peripheral blood and breast milk MTB-specific T-cell responses at 6 weeks postpartum.

Rationale and conceptual framework for immunologic hypotheses: A major goal of our application is to evaluate immune response to BCG and susceptibility to MTB infection in HEU children. In studies of adults, acute HIV is associated with a rapid decrease in MTB-specific TH1 cells.[33] MTB-specific CD4 T-cells are preferentially infected with HIV and depleted.[34] Studies suggest that BCG-specific T-cell responses are lower in HIV-infected infants, but not older children.[35,36] Intrauterine exposure to HIV may alter immune responses in HEU infants.[29,95,96] A recent study found that HEU infants have higher BCG-induced T-cell proliferation, but a lower percentage of polyfunctional T-cells compared to unexposed infants.[27] **However, there are no studies addressing whether BGC-immune responses in HEU are associated with MTB infection. To address these gaps, we will determine whether infant peripheral blood and maternal breast milk and peripheral blood immune responses are associated with MTB infection in infants.** *We hypothesize that intrauterine HIV exposure compromises the adaptive immune response to BCG vaccination with development of less effective BCG-specific memory T-cell responses that are associated with increased susceptibility to MTB infection.* We will examine early CD4 & CD8 responses, TH1 & TH17 CD4 T cell polarization, and memory T cell maturation. *We also hypothesize the breast milk from HIV-infected mothers with a prior history of MTB infection will have MTB-specific immune responses that confer protection for infants for future MTB infection.* To determine the immune correlates of risk of MTB infection and the effects of HIV exposure on response to BCG vaccination, we will examine infant BCG-specific T-cell responses and maternal breast milk MTB-specific T-cell responses.

Immunologic Study Design and Primary Analytic Goals: After routine BCG vaccination at birth, eligible HEU infants who were enrolled and randomized at age 6 weeks to INH vs. placebo, will be followed longitudinally to assess for development of MTB infection. For the efficacy study and endpoint determination, MTB status will be assessed by a blood draw at 12 months after randomization by QFT-Plus. For the immunologic studies in Aim 3, 5 mls of blood will be obtained at enrollment into the study (which is approximately 6 weeks after BCG vaccine). We will measure infant adaptive immune responses and maternal breast milk responses as outlined below. Our primary analytic goal for this aim is to determine which of these responses are associated with susceptibility to MTB infection.

Aim 3 Analysis: To identify infant and maternal (including peripheral blood and breast milk) immune correlates of risk of primary MTB infection and assess their potential interactions with INH, magnitude of immune measures (BCG-specific T-cell responses measured by ICS, cytokine/chemokine levels in peripheral blood or breast milk at 6 weeks of age and maternal peripheral blood at enrollment will be compared between cases (who subsequently acquire MTB infection) and controls (who do not) using GEE with Gaussian link to account for clustering. We will use methods for normal data and then use non-parametric approaches or dichotomize and use binomial link GEE if data are highly skewed and cannot be transformed into a normal distribution.

Please see Appendix I regarding details of the procedures and protocols as related to the infant and maternal breast milk immunologic studies.

7.6.3 Exploratory Analyses

We will compare combined infant endpoints by RCT arm as follows: Exploratory endpoint analysis #1: Infant MTB infection (IGRA positive) or active TB (probable or definite).

Exploratory endpoint analysis #2 will combine MTB infection (IGRA positive), active TB, or

death. The proportion of infants reaching the combined endpoints in each arm will be compared using chi 2 test. In addition we will compare median magnitude of IGRA responses using non-parametric tests among all infants and among the su t with positive IGRA responses.

7.7 Data Safety and Monitoring Board (DSMB)

Our study will be monitored by the DSMB. The DSMB review will address the efficacy of isoniazid (INH) as described in the primary objectives, as well as the safety of INH, as described in Section 6. The DSMB will consist of experts in pediatric TB, biostatistics and RCT trial design.

7.8 Data Monitoring

As noted above, the study will be reviewed by the DSMB for safety and efficacy. The interim efficacy analyses will be conducted and reviewed by the DSMB at 25%, 50%, and 75% of expected total number of primary endpoint events, which are 10, 20, and 30 MTB infections.

Early stopping rules for efficacy will be guided by the O'Brien-Fleming symmetric group sequential boundaries. The Lan and DeMets implementation of the boundaries will be used to define proper nominal significance levels at the interim and final efficacy analyses (a total of 4 looks). Unless there are safety concerns, significant differences (as defined by the stopping boundaries) on primary efficacy endpoints will be required in order to terminate the study early. In addition, monthly blinded safety monitoring reports will be sent to the medical officers, and protocol chairs and co-chairs for review which will include all adverse events of Grade 3 and above.

8.0 HUMAN SUBJECTS CONSIDERATIONS

8.1 Population to be enrolled and followed

The study involves two vulnerable groups: HIV-infected women and their infants. It is important to address research questions to find interventions for these vulnerable populations. The study directly addresses a research need specifically relevant to HEU infants – with the aim of optimizing prevention of TB in these infants.

8.2 Study design

We propose a randomized clinical trial of INH versus no INH. Currently, there is equipoise regarding use of IPT in HEU with conflicting results of prior IPT studies in infants, including HEU infants. IPT is not recommended during the first year of life for prevention of active TB following lack of benefit in a large RCT in South Africa. However, it is not known if INH could prevent MTB *infection*. The latter question is addressable in a Phase II RCT to evaluate impact of INH on MTB infection during the first year of life with IGRA assays to detect MTB infection.

8.3 Approvals

The study will be reviewed at Institutional Review Board at University of Washington and at the Ethical Review Committee at Kenyatta National Hospital prior to any human subjects participation. In addition, we will obtain approval from the Kenyan Ministry of Health and National Pharmacy and Poisons Board.

8.4 Informed consent

Study nurses will discuss the study rationale, design, and risks and benefits. Eligible women will be provided written informed consent prior to the participation of themselves and their infants in this study.

Risks:

INH: The study will involve provision of INH to HEU infants in the INH arm of the RCT. INH has been safe in prior RCTs and is administered routinely to TB-exposed infants. There have been concerns regarding hepatotoxicity with INH but this is a rare outcome and routine liver function monitoring is not recommended during INH in Kenya.

Phlebotomy: All infants will have blood drawn on enrollment at 6 (+/- 4) weeks of age, after 1 month of enrollment (10 +/- 4 weeks of age) and at one year following enrollment (5 mls per blood draw). Infants will also have blood drawn at 24 months of age as part of extended follow-up (5-10 mls). Infants randomized to INH will also have blood drawn for LFTs on enrollment and at 1 month after INH initiation. All mothers will have blood drawn on enrollment (5 mls). Venipuncture can cause bruising, pain, and discomfort. Blood will be drawn by an experienced phlebotomist. The amount of blood volume and frequency of blood draws is relatively small.

Breastmilk collection: Mothers will be asked to provide self-expressed breast milk at the enrollment visit. Self-expression of breast milk can cause mild breast discomfort.

Confidentiality: All mothers participating in the RCT are HIV-infected and will already be in the PMTCT program. Study data and information about HIV status will be kept confidential. Data will be stored in a locked cabinet and identifiers will not be on study records or database.

Specimen Storage for Future Studies: Specimens collected (infant blood, stool, hair, and maternal blood and breast milk) will be stored for future studies. Samples to be used in future studies will be labeled with a de-identified patient number. Samples will be kept for 10 years

after the study is complete. We will ask the Kenyatta National Hospital/University of Nairobi Ethical Review Committee and the University of Washington IRB to use of these samples in future studies prior to any new sample analysis. Caregivers who do not want to have their or their child's samples stored for future research, can still be in this study. And their samples will be destroyed once testing for this study is completed.

Benefits

Participants will benefit from direct medical care in the longitudinal research cohort.

- If INH prevents MTB infection, infants in that trial arm will benefit from INH.
- The general population of HEU and infants in general will benefit from insights gained into the tolerability and risk of SAEs associated with INH in the context of use of INH to prevent MTB infection.
- The general population of HEU and infants will benefit from insights gained into mechanisms of immune protection that can inform new prophylaxis or vaccine strategies.

Monitoring Plan

We will convene an external Data Safety and Monitoring Board (DSMB) which will be convened prior to study initiation and at 25%, 50%, 75% and 100% of expected study endpoints. If accrual is slower or the event rate of MTB infection is lower than expected, the DSMB will meet at 6 months post study initiation to review data in open and closed report, and to identify whether changes will need to be made to the recruitment plan. The DSMB will include an expert in pediatric TB, statistician, and clinician. The study will include weekly summary and expedited reporting of severe adverse events.

Extended follow-up at 24 months of age: Participants in the study will go through an additional consent for a follow-up visit at 24 months of age. If participants have exited the study prior to

consent, they will be re-contacted and given the option to consent to extended follow-up at 24 months.

8.5 Study Discontinuation

The study also may be discontinued at any time by the Thrasher Research Fund, Data Monitoring Board, and/or site IRBs/ECs.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Laboratory Specimens

Infant blood samples and maternal breast milk and peripheral blood will be collected at enrollment. These samples will transported via portable incubator to either KEMRI/CDC or CRC/CDC lab at Kisumu. These samples will be for be stimulated via the SATVI protocol and then cryopreserved. After cryopreservation, samples will be transported to Dr. Hawn's laboratory in Seattle. Dr. Hawn's laboratory will perform assays for detection of BCG-stimulated T-cell responses. A subset of samples will be transported to Dr. Cranmer at Emory University for future ancillary studies on immune responses to BCG vaccine. Stool will be collected from infants on enrollment via swab for cryopreservation at KEMRI-CDC for potential future infant microbiome studies. Blood will be drawn from infants at enrollment (6 weeks age) and 1 month post-enrollment (10 weeks of age visit) for plasma and PBMC separation. Blood will be drawn at baseline (6 weeks of age), and 1 month post INH initiation (10 weeks of age) for LFTs in infants randomized to receive INH. Baseline samples will also be used to determine INH acetylator status by testing for NAT2 genotypes. Blood will be drawn from mothers on enrollment for PBMCs and plasma separation. Maternal breast milk will be collected at enrollment for breast milk cell (BMC) and supernatant separation. A small thatch of hair (approximately 30 strands) will be cut and collected for INH exposure assessment. Hair analysis will occur at the University

of California at San Francisco Hair Analytical Laboratory (HAL) (http://test.hairlab.ucsf.edu/). The infant 5 ml blood 12-month specimen will be collected for IGRA into a single blood collection tube containing lithium heparin. These blood collection tubes will be maintained at room temperature and transported by courier to the KEMRI/CDC lab for further processing. At the KEMRI/CDC lab this blood will be transferred into QuantiFERON-TB Gold In-Tube Plus (QFT-Plus) assay collection tubes (nil, mitogen, TB antigen 1 [ESAT-6 and CFP-10 CD4 peptides], TB antigen 2 [ESAT-6, CFP-10 CD4 and CD8 peptides]) and processed per manufacture recommendations.[89] Blood will be drawn from infants at the time of any TB disease diagnosis, or at the time of study withdrawal to assess MTB infection status using the same QFT-Plus assay. In the event blood collected is insufficient blood volume (<4ml) for QFT-Plus, the blood collected will be processed for a different test for M. tuberculosis infection. Urine will be collected throughout the study from infants in the INH arm to test for INH metabolites as a measure of adherence.

Extended follow-up at 24 months: Blood will be drawn from infants at 24 months of age for plasma and PBMC separation to assess MTB infection status.

Each study site will adhere to standards of good clinical laboratory practice, and local standard operating procedures for specimen management including proper collection, processing, labeling, transport, and storage of specimens.

9.2 Quality Control and Quality Assurance Procedures

The KEMRI/CDC lab has an internationally accredited TB immunology lab that undergoes routine monitoring for QA purposes. The CDC/CRC lab also has international accreditation and undergoes routine monitoring for QA purposes. The Hawn lab has extensive experience in processing human samples for immunology studies including international trials.

9.3 Specimen Storage and Possible Future Research Testing

The KEMRI/CDC or CDC/CRC lab will store all samples while in Kenya for the duration of the study. After shipment to the US, samples will either be stored at the University of Washington, Fred Hutchinson Cancer Research Center, Emory University, or the University of California San Francisco. If patients consent at the beginning of the trial, samples will be stored after study completion for potential further study and analysis. Samples of patients who do not consent for long-term storage and additional analysis will be destroyed at study completion.

9.4 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the United States Centers for Disease Control and Prevention. All infectious specimens will be transported in accordance with United States regulations (42 CFR 72).

10.0 ADMINISTRATIVE PROCEDURES

10.1 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol consent form(s) will be approved by the UW IRB and University of Nairobi/Kenyatta Hospital ERC. The study will be registered with ClinicalTrials.gov and with the Kenyan Pharmacy and Poisons Board.

10.2 Monitoring Plan

We will convene an external Data Safety and Monitoring Board (DSMB) which will be convened prior to study initiation and 6-monthly to review data in open and closed report. The DSMB will include an expert in pediatric TB, statistician, and clinician. The study will include weekly summary and expedited reporting of severe adverse events.

10.2 Investigator's Records

The study site investigator will maintain, and store in a secure manner, complete, accurate, and current study records throughout the study. The investigator will retain all study records for at least three years after submission of the study results. Study records include administrative documentation — including protocol registration documents and all reports, and correspondence relating to the study — as well as documentation related to each participant screened for and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

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12.0 APPENDICES

I SCHEDULE OF STUDY VISITS AND PROCEDURES

Table 3: Brief overview of study visits and procedures

Table 3: Overview of Study Visits and planned procedures					
	6 weeks postpartum	**Follow-up visit***	**12 months post enrollment**	**TB Diagnosis**	**24 months of age**
HIV testing (per MOH)	**x**	**x****	**x**		
Enrollment	**x**				
Sociodemographic survey	**x**	**x**	**x**		
Health history	**x**	**x**	**x**		**x**
Physical exam	**x**	**x**	**x**		**x**
TB (infant and maternal) symptom screen	**x**	**x**	**x**		**x**
SAE assessment		**x**	**x**		
Adherence assessment via questionnaire, urine INH testing***		**x**	**x**		
TB exposure assessment	**x**	**x**	**x**		**x**
Infant blood draw	**x**	**x****	**x*****	**x*****	**x**
Maternal blood draw	**x**				
Maternal breastmilk collection	**x**				
Infant stool collection	**x**				
Infant TST placement			**x*****	**x*****	**x**
Infant hair collection***			**x**		

* Follow up visits will occur at 10 and 14 weeks of age, and 6, 9, and 12 months of age.
** Infant DNA PCR will be drawn a 6 weeks of age and HIV antibody test will be drawn at 12 months of age per Kenyan MOH guidelines.
*** For infants randomized to INH
****For all infants blood will be drawn for PBMCs and plasma at the 10 week of age visit. For infants randomized to INH arm, LFTs will be drawn at baseline (6 weeks) and 10 weeks of age (1 month post INH inititation).
***** Blood will be drawn to assess the presence of Mtb infection at study endpoint,time of TB diagnosis, and in the event of study withdrawal using QFT-plus.TST will also be placed and read within 48-96 hours. If blood volume is insufficient for QFT-Plus (<4ml), blood will be processed for for a different test for M. tuberculosis infection.

Table 4: List of study visits and procedures (details on laboratory assays, infant adaptive immune response assays, and maternal breast milk TB specific cellular immune responses assays will be detailed below the table)

Table 4: Detailed study visits and procedures		
Visits and procedures		**Details**
2 weeks postpartum	Informed and invited	HIV-infected mothers with HEU infants will be informed about the study starting from 2 weeks postpartum and will be invited to enroll their infant in the study.
2 - 10 weeks	Recruitment	Written informed consent will be obtained before any study procedure.
6 (+/- 4) weeks of age	Enrollment, screening, and randomization	*Enrollment and screening:* • HIV DNA PCR testing will be used to confirm HIV negative status. • Infant blood (5 ml) will be collected for baseline PBMC separation and WBA assays, and NAT2 genotype polymorphisms associated with INH acetylation phenotypes. • Maternal breast milk (30 ml) will be collected. • Maternal peripheral blood (5 ml) will be collected. • Growth measures, mid-upper arm circumference, infant feeding, and symptoms (including cough and fever), and presence of BCG scar will be assessed. • Infant stool will be collected for cryopreservation for future infant microbiome studies • Household locator information, HIV care medical identification number, and cell-phone contacts will be obtained to facilitate tracing. • Infant adaptive immune response and maternal breast milk TB specific cellular immune responses will be determined (detailed below). *Randomization:* • Block site-stratified randomization will be used to allocate infants 1:1 to INH or no INH trial arms. Randomization numbers will be generated at UW prior to study start (under leadership of the Study Biostatistician and with CFAR Biostatistical Core).
Daily	Intervention	Isoniazid ~10 mg/kg (7-15 mg/kg) and pyroxidine (1-2 mg/kg) will be administered once daily to infants in INH arm for 12 months.
Follow-up visits (10 weeks, 14 weeks, 6 months, 9 months, 12 months of age)	INH pick-up; assessment of infant morbidity and adherence; TB symptom screening of mother and infant.	• At follow up visits intercurrent infant morbidity will be evaluated using standardized questionnaires. • Both mothers and infants will be evaluated with standard TB screening questions regarding their own and household TB exposures. Any mother or infant with suspected active TB will be referred for TB microbiologic testing and X-rays, and these results will be abstracted to the study database. Mothers with suspected active TB will be offered sputum AFB and GeneXpert testing consistent with Kenyan Ministry of Health guidelines. Infants with suspected TB will have chest X-ray, gastric aspirate testing by GeneXpert, and clinical review and classification as definite, probable or possible TB using Graham and NIH/WHO 2014 criteria. • Adherence will be assessed using maternal report, pill counts at re-fill visits, urine INH dipstick testing (for infants in INH arm) • Blood will be drawn from all infants for PBMCs and plasma at the 10

Follow-up visits (cont)		week of age visit. • LFTs will be drawn at baseline (6 weeks) and 1 month post initiation (10 weeks) for infants INH randomized to receive INH
	Maternal ART visits.	Maternal medical records will be abstracted to obtain data on maternal ART regimen, cotrimoxazole status, CD4 and viral load (if available). Anticipated maternal regimen will be tenofovir, efavirenz, emtricitabine (or lamivudine) with 6 weeks of infant nevirapine postpartum.
At 12 months post randomization		• Infant blood will be drawn for IGRA to ascertain potential MTB infection. • Infant HIV status will be determined at exit using repeat HIV DNA PCR testing at CDC-KEMRI laboratories to confirm that infants remain HIV uninfected. • TST will be placed and read within 48-96 hours • Infant hair will be collected at the study endpoint for INH levels for children in the INH arm
At 24 months of age		• Infant blood will be drawn for plasma and PBMC separation • Infant adaptive immune responses will be determined (detailed below) and will include ascertainment of potential MTB infection • TST will be placed and read within 48-96 hours • Intercurrent infant morbidity will be evaluated using standardized questionnaires.

Laboratory Assays: At baseline, infant blood will be collected and stimulated using SATVI protocol and transported in a portable incubator to the CDC-KEMRI lab in Kisumu prior to cryopreservation and transport to Dr. Hawn's laboratory for detection of BCG-stimulated and ESAT-6 and CFP-10 stimulated responses. This SATVI protocol was developed for use in this type of clinic, which is linked to a centralized laboratory. Blood will be drawn on enrollment from mothers on enrollment for PBMCs and plasma. Maternal breast milk will be collected at enrollment for BMCs and supernatant. All infants will have blood drawn at enrollment and at the 10 week of age visit for plasma and PBMCs. Infants that are randomized to receive INH will have blood drawn on enrollment and 1 month post INH initiation for LFTs. Baseline samples will also be used to determine INH acetylator status by testing for NAT2 genotypes. The 12-month infant 5 ml blood specimen will be collected into a single lithium heparin blood collection

tube and kept at room temperature until transported to KEMRI/CDC. At KEMRI/CDC blood from the single collection tube will be transferred for IGRA directly into QFT-Plus assay collection tubes (nil, mitogen, TB antigen 1, TB antigen 2) and processed per manufacture recommendations.[89] The assay measures the amount of interferon-gamma (INF-γ) released by primarily CD4+ T helper lymphocytes after stimulation with TB-specific antigens (ESAT-6, CFP-10 and TB7.7) to measure MTB infection as well as the INF-γ released by CD8+ cytotoxic T lymphocytes after stimulation with the same TB-specific antigens. A response of ≥0.35 IU/ml to the TB antigens in either TB 1 or TB 2 (with Nil < 8 IU/ml and positive mitogen control) will be considered a positive result. Blood for QFT-Plus assay will also be drawn in the event of an infant TB diagnosis, or study withdrawal. In the event blood collected is insufficient blood volume (<4ml) for QFT-Plus, the blood collected will be processed for a different test for M. tuberculosis infection. A small thatch of hair (approximately 30 strands) will be collected from children in the INH arm at the 12 months post randomization study endpoint visit (approximately 14 months of age) to measure INH levels.

Infant Adaptive Immune Response Assays: We will determine several T-cell characteristics as follows:

A. <u>Frequency, cytokine profile, and effector/memory/homing/activation phenotype of mycobacterial-specific CD4+ and CD8+ T cells after short term incubation with a flow-cytometric intracellular cytokine assay.</u>

We will use multi-parameter flow cytometry with ICS using whole blood from the baseline enrollment time-point in cases and controls. We will use a short-term assay (7 & 12 hours) with 200 ul of blood per condition with several stimuli including: 1. live BCG; 2. peptide pools of

MTB antigens CFP-10/ESAT-6 (to exclude individuals with immune responses to MTB infection rather than BCG); and 3. controls (medium alone and PHA). This assay was developed at SATVI and utilizes a 37°C incubator which can be deployed at clinic sites and used for transport to the reference laboratory. This protocol was used successfully with field site utilization for processing 5,724 samples in an infant BCG trial [69]. Tubes are pre-coated with anti-CD28 and anti-CD49d, incubated with blood for 7 hours with removal of supernatant, incubated an additional 5 hours with Brefeldin-A before harvesting and fixing with FACS lysis buffer and cell cryopreservation.

Immune Measures: Frequency of single and combined expression of IFN-γ, IL-2, TNF-α,IL-17 and IL-22 in viable CD4 and CD8 T cells; expression pattern of HLA-DR, CD38, CD45RA, CCR7, CXCR3, α1 and β4 in viable cytokine+ (i.e., specific) CD4 and CD8 T cells; and expression patterns of PD-1, CTLA-4, CD160, and FoxP3 on/in these cells.

B. CD4+ and CD8+ T cell proliferation, survival, and differentiation and expression of cytotoxic markers after longer term incubation: PBMCs, pre-stained with Ki-67, will be incubated with BCG, CFP-10/ESAT-6, and control conditions for 3 or 6 days, and then fixed and stained for CD3 and CD8. The short-term incubation (7 hours for secreted cytokine and 12 hours for ICS) measures a quantitative ex vivo snapshot of immunity, before cells are able to proliferate. In contrast, the longer term assays may evaluate distinct aspects of immunity (e.g., central memory cells), and allow detection of some markers not optimally measurable in the mycobacterial system with short term assays (e.g., cytotoxic markers, type 2 cytokine responses). Ki-67 is an excellent marker of specific cells in these assay systems, and its expression on day 6 correlates

with traditional markers of proliferation such as BrdU and CFSE dilution79. The pattern of expression of cytotoxic markers may correlate with distinct cellular functional attributes. Further, direct measurement of cytotoxicity is impractical, given available PBMC numbers. We will use several stimuli including: 1. live BCG; 2. Peptide pools of MTB antigens CFP-10/ESAT-6 (to exclude individuals with immune responses to MTB infection rather than BCG); and 3. Controls (medium alone and PHA).

Immune Measures: On day 3, frequency of single or combined expression of granulysin, granzyme B and perforin in viable, Ki67+ (i.e., antigen-specific, proliferating) CD4 and CD8 T cells. On day 6, absolute numbers and frequency of viable CD4 and CD8 T cells; frequency of viable Ki67+ CD4 and CD8 T cells; expression pattern of IFN-γ, IL-2, TNF-α, IL-17, IL-4, IL-13 and IL-10 in viable Ki67+ CD4 and CD8 T cells.

C. Secreted T cell cytokines in stimulated whole blood: We will assess soluble production of T cell cytokines at 7 hours and at 6 days, focusing on cytokines not readily detectable by the assay systems above, in supernatants, with bead arrays. *Immune Measures*: We will measure levels of 29 cytokines/chemokines measured by multiplex bead array technology (which includes IL-2, IL-4, IL-5, IL-10, IFN-γ, TNF-α, and TGF-β).

Hair analysis for INH exposure: A small thatch of hair (approximately 30 strands) will be cut from the occiptital region close to the scalp, place in tin foil, sealed inside a plastic bag containing desiccant, and then stored at room temperature before being shipped to the UCSF Hair Analytical Laboratory (HAL). INH will be extracted from hair cut samples via methanol/water solution (v/v, 8/2) containing 1% hydrazine dehydrochloride, followed by

evaporation and reconstitution prior to separation by liquid chromatography/tandem mass spectrometry. Extracted sample analysis will be performed by mass spectrometer using positive ionisation. The assay has been validated over the linear dynamic range of 0.5–100 ng INH/mg of hair utilising 20–30 strands of human hair (~1–3 mg).

Table 5: Sample Collection Schedule & Volumes

Time	Blood	Breast milk	Stool	Hair	Assay
6 wk	Infant: 5 mls (immunologic assays) + 2 mls (LFTs) Mothers: 5 mls	30 mls	< 5ml (swab)	NA	BCG induced T-cell profiling, PBMCs and plasma for innate & T-cell assays; ESAT-6 and CFP-10 IGRA in PBMCs and breast milk cells. Assays will include a whole blood cytokine assay performed at the time of the blood draw. In addition, PBMCs and plasma will be cryopreserved and examined later with assays that include stimulation with BCG and CFP10/ESAT6 with analysis by flow cytometry, as well as determination of NAT2 genotype for acetylator phenotype. Stool collected by swab will be cryopreserved for potential future infant gut microbiome studies. For infants randomized to receive INH, blood will be drawn for LFTs at baseline.
10 wk	5 mls (immunologic assays) + 2 mls (LFTs)	NA	NA	NA	PBMCs + plasma for storage for future exploratory studies including role of antibodies and infant MTB infection, role of IPT on BCG response For infants randomized to receive INH, blood will be drawn for LFTs at 1 month post INH initiation.
12 mo post randmonization	5 mls	NA	NA	Approx 30 strands	Infant IGRA using QFT-Plus For infants randomized to receive INH,hair analysis for INH exposure
TB diagnosis, Study withdrawal	5 mls	NA	NA	NA	Infant IGRA using QFT-Plus (In the event blood collected is insufficient blood volume (<4ml) for QFT-Plus, the blood collected will be processed for a different test for MTB infection.)
24 mo	5 mls	NA	NA	NA	PBMCs and plasma for innate & T-cell assays; ESAT-6 and CFP-10 IGRA in PBMCs.

Analysis of Adaptive Immune Measures and Association with MTB infection: Using the immune measures outlined in A-C above, BCG-specific immune measures at the enrollment visit will be compared in HEU infants who later develop MTB infection and those who remain MTB uninfected at 12 months following enrollment. For these comparisons, infants with evidence of baseline CFP-10/ESAT-6 responses will be excluded. We will examine whether BCG-specific

cytokine expression (by ICS or ELISA) is associated with the development of MTB infection. We will assess several T-cell characteristics including: 1. T-cell subtype (CD4 vs CD8), 2. polyfunctionality of CD4 effector phenotype (assessing IFN-γ, IL-2, and TNF), 3. TH subset polarization with assessment of at least TH1 and TH17 subsets, and 4. central and effector memory subtypes (CD45RA/RO, CCR7). In addition, we will assess a recently described memory T-cell phenotype (CXCR3+CCR6+) that was found to be a dominant MTB-specific phenotype in the genome wide screen of CD4 epitopes in LTBI subjects.[97] *A priori* we hypothesize that 6-week BCG-specific CD4 IFNγ, CD8 IFNγ, and/or polyfunctional responses will be less frequent, and that there will be fewer central memory ($CD45RA^{-}CCR7^{+}$) cells among infants who later acquire MTB infection than in those with no evidence of MTB infection, matching for age at assessment of IGRA status, and any reported TB exposure. To assess whether INH modifies BCG responses, we will compare BCG immune responses at 12 month follow-up in a randomly selected subset of 20 infants from each trial arm.

Maternal Breast Milk TB Specific Cellular Immune Responses

In addition to testing infant peripheral immune responses to BCG, we will examine whether maternal immune responses in breast milk are associated with infant protection from MTB infection. In a Canadian cohort, breastfed infants had significantly enhanced cell-mediated responses to BCG compared to formula fed infants.[38] Similar to peripheral blood, there are several types of protective immune responses that could be present in breast milk including innate and MTB-specific T-cell responses. The MTB-specific T-cells could be present from prior maternal MTB infection or TB disease. The presence of these T-cells could provide protection for the infant. There are also unique molecules (e.g. lactoferrin) and potential for different

cellular trafficking and differentiation within breast milk. With our preliminary data that MTB-specific T-cell responses are present in BMCs and of higher magnitude than peripheral responses, we will examine whether these responses are associated with protection from infant MTB infection. To accomplish this sub aim, we will measure *MTB-specific T-cell responses in maternal breast milk cells and peripheral blood:* breast milk (30 mls) and peripheral blood (5ml) will be collected at enrollment and BMCs and supernatant will be isolated and cryopreserved. Cells will be thawed and stimulated with 1. Peptide pools of MTB antigens CFP-10/ESAT-6; and 2. controls (medium alone and PHA). We will use flow cytometry and ICS to measure a panel of CD4 and CD8 T-cell responses as described above.

II ISONIAZID DOSING

Table 6: Weight-based dose of isoniazid to be used in study using based on WHO and Kenya MOH national guidelines.

Table 6: Dose of Isoniazid (INH) for Isoniazid preventive Therapy (IPT) in children

Weight (kg)	Daily Dose in mg	Number of 100 mg tablets
<5	50	½
5.1 – 9.9	100	1
10-13.9	150	1½
14-19.9	200	2
20-24.9	250	2½
>25	300	3*

***For children more than 25 kg, one can use 1 adult tablet of INH (300mg) once daily (max 300 mg/day)**

(Source: Kenya Ministry of Health. National Guidelines on Management of Tuberculosis in Children, Second Edition. August 2013. Division of Leprosy, Tuberculosis and Lung Disease.)

III PYRIDOXINE DOSING

Table 7: Weight-based dose of pyridoxine to be used in study using based on Kenya MOH national guidelines.

Table 7: Dose of pyridoxine to be used with INH administration		
Weight (kg)	**Daily Dose in mg**	**Number of 50mg tablets**
5-7	12.5	1/4
8-14	25	1/2
15 and above	50	1

(Source: Kenya Ministry of Health. National Guidelines on Management of Tuberculosis in Children, Second Edition. August 2013. Division of Leprosy, Tuberculosis and Lung Disease.

IV SAE TABLES SPECIFIC TO PERIPHERAL NEUROPATHY

Table 8: Supplemental toxicity table for grading severity of peripheral neuropathy in children

GRADE	SYMPTOM
Grade 2	Unable to do one or more upper or lower extremity age-appropriate task on truncated Denver Developmental test OR Conveys that there is mild pain or burning sensation in hands or feet by expressing discomfort on touch or pressure of extremity or by refusal to use extremity, but has normal ankle and knee reflexes, muscle bulk, tone and strength.
Grade 3	Unable to do any upper extremity or lower extremity age-appropriate tasks on truncated Denver Developmental test OR Conveys pain or burning sensation in hands or feet by expressing discomfort on touch or pressure of extremity or by refusal to use extremity AND Ankle reflexes are hypoactive or absent but knee reflexes are normal
Grade 4	Unable to do any upper extremity or lower extremity-age appropriate tasks on truncated Denver Developmental test OR Conveys that pain or burning sensation exists in hands or feet by expressing discomfort on touch or pressure of extremity or by refusal to use extremity AND Either: (1) ankle *and* knee reflexes are hypoactive or absent, or (2) muscle bulk, tone or strength is decreased, or (3) foot drop is present

Table 9: Supplemental toxicity table for grading severity of peripheral neuropathy in children Evaluation of peripheral Neuropathy using truncated Denver Developmental test. Participants should be able to pass age-appropriate evaluations listed below. Performance rated as "Yes, No, or Unable to assess (subject not cooperative)."

AGE	EVALUATION
3-4 months	Tests for peripheral neuropathy in upper extremities: • Grasp rattle • Put hands together Tests for peripheral neuropathy in lower extremities • Bear weight on legs
6 months	Tests for peripheral neuropathy in upper extremities: • Pass a cube from hand to hand • Rake a bead Tests for peripheral neuropathy in lower extremities • Bear weight on legs
9 months	Tests for peripheral neuropathy in upper extremities: • Thumb finger grasp • Bang two cubes together Tests for peripheral neuropathy in lower extremities • Stand holding on
12 months	Tests for peripheral neuropathy in upper extremities: • Put block in cup • Bang two cubes held in hands Tests for peripheral neuropathy in lower extremities • Stand two seconds

V Participant retention of previous RCT studies in Kenya by study staff

Table 10: Previous RCTs conducted by study team			
Study, Lead Investigators (publications)	**Cohort size**	**Retention %, duration**	**Finding**
1. Breast versus formula feeding *Nduati, Kreiss (JAMA 2000, 2001, Lancet 2000)*	425 m-i pairs	94% 2 year	Risk of breastmilk HIV transmission
2. Rapid testing for PMTCT *Malonza, John-Stewart (AIDS 2003)*	1249 preg women	NA	Rapid HIV test superior
3. PMTCT compliance *Kiarie, John-Stewart (AIDS 2003)*	139 m-i pairs	84% 6 wk	Comparable SC PMTCT adherence
4. ZDV effect on genital HIV *Mbori-Ngacha, John-Stewart (J Virol 2003)*	42 preg women	100% 1 wk	Rapid decline in genital HIV RNA
5. NVP vs. ZDV PMTCT *Chung, John-Stewart (AIDS 2005)*	66 m-i pairs	85% 6 wk	NVP longer HIV suppression in BM
6. ZDV/NVP vs. HAART PMTCT *Chung, John-Stewart (Antiviral Therapy 2006)*	58 m-i pairs	85% 1 yr	BM RNA but not DNA decline
7. Valacyclovir to decrease HIV *Drake, Farquhar (J Infect Dis 2012)*	148 m-i pairs	94%, 12 mos	VCV decreases plasma HIV RNA
8. Diary for pediatric adherence *Wamalwa, John-Stewart (JIAS 2009)*	90 children	84%, 15 mos	Diaries do not improve adherence
9. ART counseling vs. alarm *Chung, John-Stewart (PLoS Med 2011)*	400	87%, 18 mos	Counseling superior
10. Partners HSV *Study PI Celum; Nairobi Site PIs: John-Stewart, Kiarie, Farquhar (NEJM 2010, Lancet 2011)*	3408 couples 416 Nairobi	84% uninf, 92% inf, 2 yr	HSV-suppression does not decrease HIV transmission
11. Partners PrEP *PI Baeten, Celum; Nairobi Site PIs: John-Stewart, Kiarie, Farquhar (NEJM 2012)*	4747 couples, 485 in Nairobi	96%, 2 years	PrEP decreases sexual transmission
12. Albendazole in HIV, helminth-infected adults *Walson, John-Stewart (AIDS 2009)*	208	97%, 3 month	Increased CD4 post-albendazole in ascaris
13. Program Helminth Eradication *Walson, John-Stewart (Lancet Inf Dis 2012)*	948	95.5%, 2 years	Deworming does not change HIV progression
14. Optimizing pediatric ART *Wamalwa, John-Stewart (CROI 2012)*	42	98%, 18 months	Early ART with PI safe but not durable
15. CTX cessation post-ART *Polyak, John-Stewart (CROI 2014)*	500	98%, 1 year	CTX cessation increased malaria
16. Partner HIV testing in PMTCT *Osoti, Farquhar (AIDS 2013)*	300	99%, 6 weeks	Home-based partner testing effective
17. Pediatric HIV vaccine *Hanke, Jaoko, John-Stewart (Vaccine 2014)*	72	99%, 48 weeks	MVA-HIVA safe but not immunogenic
18. Mobile WACh for MCH *Unger, John-Stewart*	300	Ongoing	Ongoing, 300 enrolled; in follow-up
19. Urgent ART for hospitalized children *Wamalwa, John-Stewart*	360	Ongoing	Ongoing, >120 children enrolled

VI HAIR COLLECTION PROTOCOL FOR ANALYSIS OF INH EXPOSURE

Hair collection protocol

Materials required: Scissors, piece of tin foil, patient labels (2), ziplock bag, alcohol swabs, and desiccant pellet

Suggest making these "hair kits" ahead of time

Step 1: Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry

Clean off blades of scissors between patients

Step 2: Lift up the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (~30 fibers of hair) from underneath this top layer

Can use hair clip to keep top layer of hair away if easier

Step 3: Cut the small hair sample as close to the scalp as possible

STRAIGHT HAIR

CURLY HAIR

SHORT HAIR

Can let hair fall directly into piece of tin foil when very short/cropped (no need to label end since too short)

BRAIDED HAIR

Cut hair thatch from in-between braids or dread locks

Step 4: Keep your fingers on the part of the hair that was FURTHEST away from the scalp and put the hair sample down on an unfolded piece of tin foil

Step 5: Put a thin label over the end of the hair sample that was FURTHEST away from the scalp

If hair very short just let it fall into the piece of tin foil and no need to label the distal end

Step 6: Refold the foil over to completely enclose the hair and place a study ID label on the folded piece of foil

Step 7: Place the folded piece of foil inside the plastic (e.g. Ziplock®) bag (desiccant pellet in the bag is optional) and seal the bag;

Good collection: Distal end (side farthest from scalp) labeled

Bad collection: Distal end could have been labeled (long enough) but not

Okay not to label because too short

Hair samples should be kept at room temperature and in a dark place at each site prior to batch shipment (without biohazardous restrictions) to our hair laboratory at UCSF.