Preventing Mycobacterium Tuberculosis Infection in HIV-Exposed Infants

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

Statistical Analysis Plan

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1. STUDY SUMMARY AND AIMS

Rationale: HIV-exposed uninfected infants (HEU) in HIV/TB endemic settings have a high risk of *Mycobacterium tuberculosis* (MTB) infection and TB disease, even in the absence of known MTB exposure. [1, 2] Because infancy is a time in which there is rapid progression from primary to active TB[3], 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 isoniazid preventive therapy (IPT) prevents primary MTB infection in HEU infants, to determine timing and cofactors of primary MTB acquisition in the first year of life, and to 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.[4, 5] Only one of these evaluated HEU infants, and found no protective effect of IPT in decreasing TB disease.[4] 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 interferon gamma release assays (IGRAs), corresponding to a 20% annual cumulative incidence of infection.[6] This suggests that HEU infants have a substantial incidence of MTB infection related to community and household TB exposure. There are no longitudinal studies of MTB infection among HEUs using serial IGRA testing. Unlike tuberculin skin testing (TST), 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.

Design: A 2-arm, non-blinded randomized clinical trial (RCT) comparing the efficacy of a 12 month course of isoniazid (INH) vs. no INH to prevent *M. tuberculosis* infection among HIV-exposed uninfected Kenyan children aged enrolled at 6 weeks of age.

Additional nested sub-studies will evaluate epidemiologic and immunologic correlates of MTB infection, and to explore the role of INH in prevention of active TB and mortality.

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

Sites: Kisumu County Hospital (KCH) in the Nyanza province of Kenya with additional sites in Ahero and Bondo if necessary for recruitment.

Sample Size: 300 children will be randomized (150/arm)

Duration of follow-up: 12 months (enrollment at 6 (+ 4) weeks of age, 10 & 14 weeks of age, 6 & 9 & 12 months of age, and 12 months post study enrollment)

Primary Objective:

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 or tuberculin skin test (TST) 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.

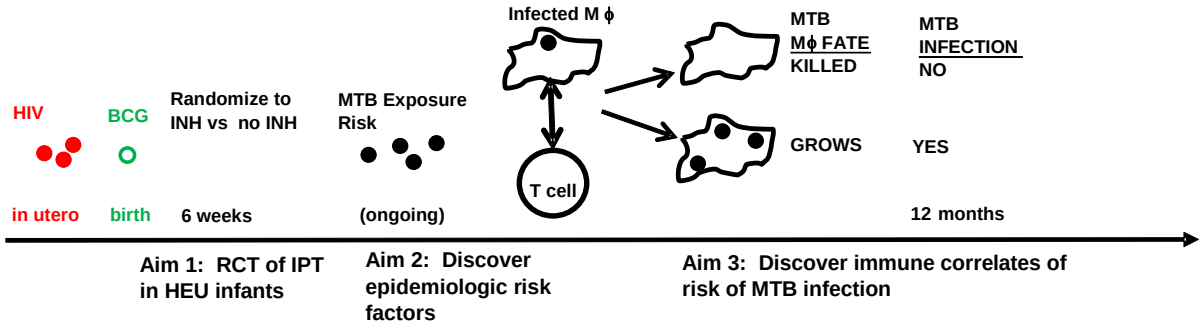

Figure 1: Aims of RCT to evaluate INH to prevent MTB infection in HEU infants

2. STUDY END-POINTS

Primary study end-points:

1. **MTB infection** will be assessed by interferon gamma release assays (IGRA, QFT-Plus) or TST at 12 months post-enrollment. QFT-Plus assay will be run at KEMRI/CDC Kisian TB immunology lab.

Secondary end-points:

2. **Severe Adverse Events (SAE):** number of infants with grade 3 or higher treatment-related adverse events as assessed by DAIDS Table for the Grading Severity of Pediatric Adverse Experiences.

3. **Combined outcome of MTB infection including IGRA, TST, and additional interferon-gamma-independent immune markers in QFT-Plus supernatants**. Combined outcome will be defined as positive if IGRA OR TST OR interferon-gamma-independent marker is positive and combined outcome will be defined as negative if none of these is positive (if children do not have all three markers the definition will hold for available markers).

4. **Epidemiologic correlates of MTB infection** will be assessed via structured questionnaire and medical record review, by collecting information on the following:

- 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

5. **Immunologic correlates of MTB infection** will be assessed via:
    - 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.

Exploratory end-points:

1. **A combined endpoint of MTB infection, TB disease, and death** will be assessed via:
    - MTB infection as measured by IGRA or TST at 12 months post-enrollment
    - TB disease including microbiologically confirmed (culture or Xpert positive), or probable TB (clinical diagnosis)
    - Death of HEU infant

3. SAMPLE SIZE CONSIDERATIONS

The primary endpoint will be a MTB infection measured by IGRA (QFT-Plus). Assuming alpha=5%, power=80%, 2-sided test, and a 1:1 allocation ratio, 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

4. STATISTICAL ANALYSES

4.1 Intention to treat

Analysis of primary outcomes will be by intention-to-treat (all HIV-exposed uninfected participants).

4.2 Study accrual

Per CONSORT guidelines, we will report the number of individuals who (See Figure 2a):

1. Underwent screening
2. Met inclusion criteria

3. Did not meet inclusion criteria (and reasons)
4. Enrolled in the study and were randomized
5. Treated as per study protocol (received at least one of study drug in INH arm, and did not receive INH in the No INH arm)

The number of individuals enrolled and randomized including visit completion will be described by arm. No formal statistical testing will be performed at interim DSMB reviews for enrollment or randomization. Loss to follow-up by arm will be presented by arm for the closed DSMB report.

Figure 2a – Detailed trial profile

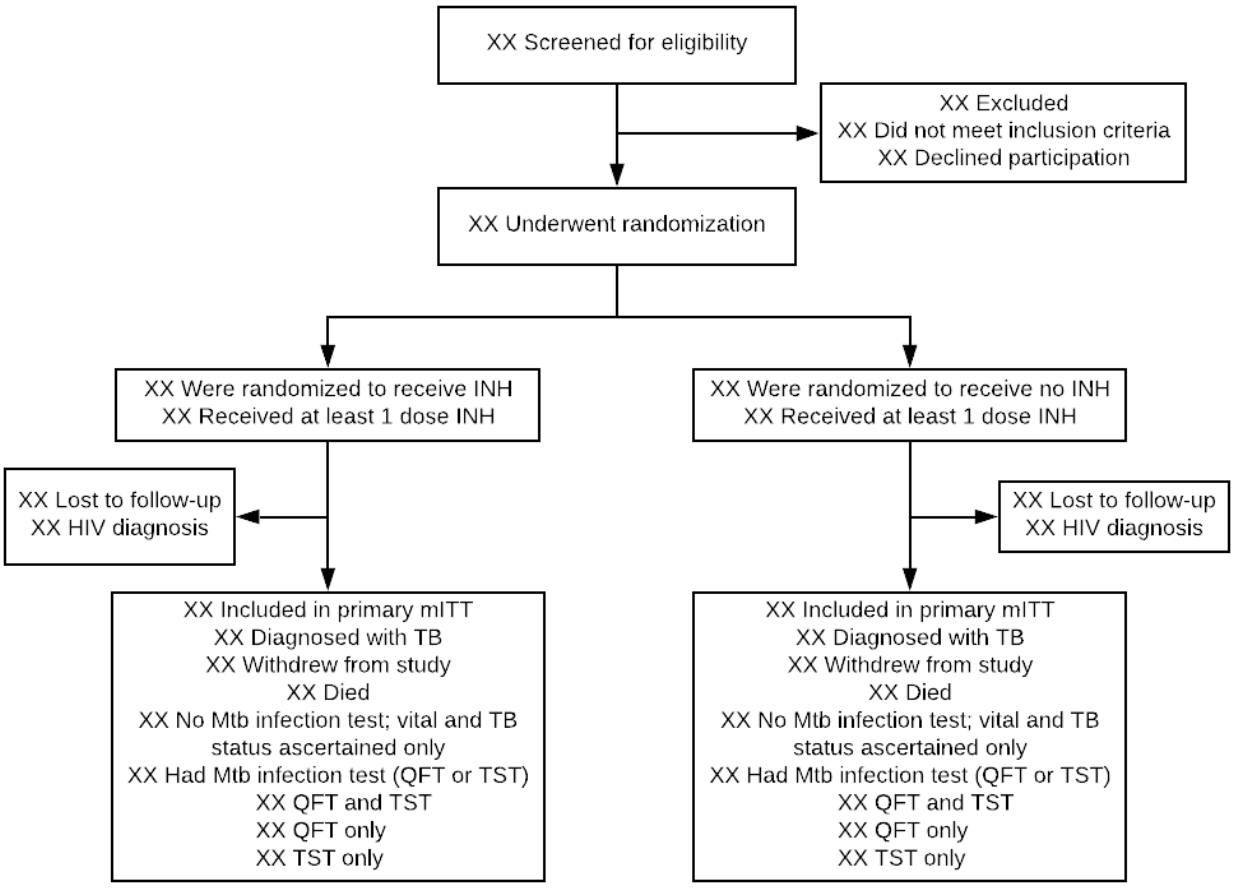

Figure 2b – Detailed Enrollment and Follow-up (Open report)

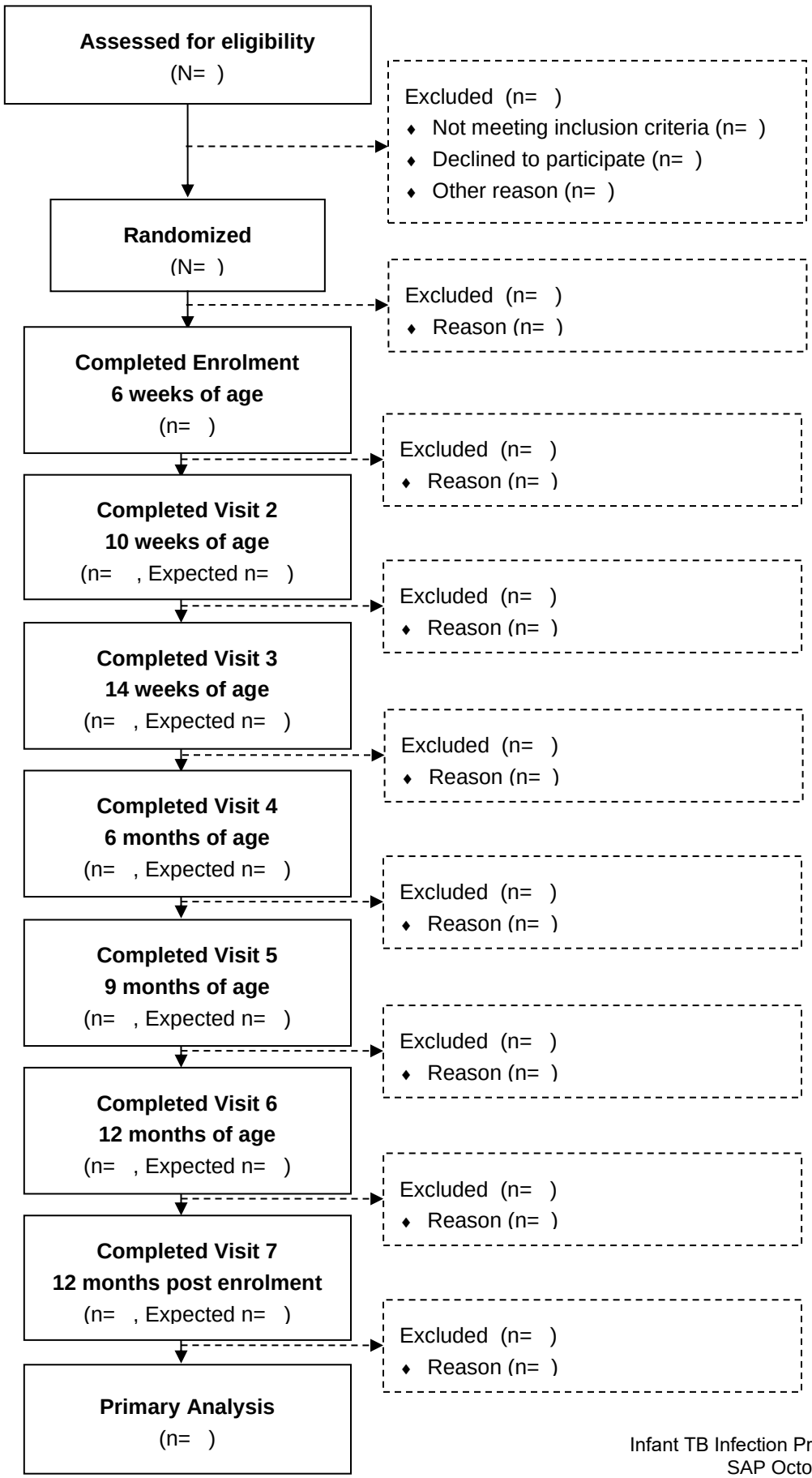

Figure 3 – Histograms depicting loss to follow-up by arm (Closed report)

A histogram depicting proportion of participants retained in the study at each visit will be provided.

4.3 Baseline Characteristics

We will describe the distribution of baseline characteristics, using summary statistics appropriate for the measurement scale. For the Closed report, this will be presented by blinded intervention groups (X,Y) (Table 1a). For the Open report, this will be presented in aggregate.

Table 1a Baseline characteristics: shell table (Closed report)

		All Participants N=		INH N=		No INH N=
Characteristic	**N**	**Median (IQR) or No. (%)**	**N**	**Median (IQR) or No. (%)**	**N**	**Median (IQR) or No. (%)**
Infant characteristics						
Sociodemographic						
Infant age (weeks)						
Weight-for-age Z score						
Underweight (WAZ < -2)						
Weight-for-age Z score among underweight						
Male						
Breastfeeding						
Ever breastfed						
Breastfed on enrollment						
Infant PMTCT/BCG						
Infant received ARVs for PMTCT						
Infant BCG vaccination						
Maternal characteristics						
Sociodemographic						
Maternal age						
Secondary education started						
Parity						
Maternal HIV/TB						
Maternal partner HIV status						*
Positive						
Negative						
Unknown						
Maternal HIV diagnosis						
Before pregnancy						
During pregnancy						
After delivery						
Maternal ARVs						
Initiated before pregnancy						
Initiated during pregnancy						
Initiated after pregnancy						
Maternal ARV regimen						

Efavirenz-based regimen

Nevirapine-based regimen

Protease inhibitor-based regimen

Maternal CD4 (cells/mm3)

Maternal HIV RNA (copies/ml)

HIV viral load >1000

Maternal history of TB

Maternal history of IPT

Initiated before pregnancy

Initiated during pregnancy

Initiated after pregnancy

Residential characteristics

Crowding

Persons in household

Rooms in household

Housing

Toilet type in household

None

Pit latrine

Flush

Roof type

Thatched

Iron sheet

Electricity in home

Running water in home

4.4 Analysis of study end-points

Note: Analysis focused on primary and safety end-points (Aim 1)

Modified intention to treat: The primary modified intention-to-treat analysis will exclude infants found to be HIV DNA positive at enrollment or during the study. ***At the guidance of the DSMB TST was added in combination with IGRA for the primary endpoint of MTB infection.***

Primary endpoint: MTB infection. We will use a Chi square (χ^2) test to compare the prevalence of infant Mtb infection (by IGRA or TST) at 12 months following enrollment between INH and No INH arms in an intent-to-treat analysis (ITT). If the baseline assessment of randomization (Table 1a) reveals an imbalance in characteristics between the treatment groups, we will evaluate these variables as potential confounders in a sub-analysis secondary to the ITT. Potential baseline confounders will evaluated individually to see if adjustment changes the odds ratio by more than 10%. Collinearity between potential confounders will be evaluated. After excluding collinear variables, these potential confounders will be added to a multivariable logistic model.

We will also evaluate the cumulative incidence of Mtb infection (By IGRA or TST) and compare the hazard ratio of MTB infection between arms. Since infants are only tested once at the end of the study and the timing of actual Mtb infection is unknown, infants with Mtb infection assumed to have become positive at end of 6 months; infants without Mtb infection at end of study censored at completion of 12 months and assumed to be negative at time of censor

Per protocol: We will also evaluate our primary outcome by a per protocol analysis, considering only HEU infants who took at least one dose as taking INH vs. infants who did not take any INH. We anticipate future sensitivity analyses using IPT adherence and continuation data as exposure of interest and Mtb infection as outcome.

Exploratory endpoint: combined endpoint of MTB infection, TB diagnosis, and death. Time to combined endpoint-free survival will be compared between randomization groups using Kaplan-Meier (K-M) survival analysis and a log-rank test in an ITT analysis.

Table 2a demonstrates how primary endpoint data will be reported. Table 2b demonstrates how secondary MTB infection endpoint data will be reported. Table 2c demonstrates how exploratory endpoint data will be reported.

Secondary endpoints:

Safety outcomes: We will compare proportions of participants with severe adverse events (Grade >3 per DAIDS Grading Severity of Pediatric Adverse Experiences) by arm using either Chi-squared or Fisher's exact tests as appropriate for $\geq$ grade 3 serious adverse events.

Secondary epidemiologic and immunologic endpoints will evaluated after the end of the study, and will not be part of DSMB review. An analysis plan for these secondary endpoints is included below for reference only.

In addition, we will conduct secondary analyses using an expanded Mtb infection definition including a positive TST, QFT-Plus, or IFN-γ-independent immune markers in QFT-Plus supernatants.

Epidemiologic correlates of MTB infection. 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.

Immunologic correlates of MTB infection. 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.

Table 2a. Primary Outcome - Prevalence of MTB infection at endpoint (mITT)

Outcome	X group (N=)		Y group (N=)		Effect Estimate		
	n	%	n	%	RR	(95% CI)	*p*-value
MTB infection*							
QFT-positive							
TST positive							

**Either QFT or TST positive

Table 2b. Primary Outcome - Cumulative incidence of MTB infection at endpoint (mITT)

Cumulative incidence of Mtb infection over 12 months*											
Primary Outcome	**Overall (N=)**			**X group (N=)**			**Y group (N=)**			**HR (95% CI)**	**p**
	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)		
MTB Infection											
	Overall (N=)			**X group (N=)**			**Y group (N=)**			**HR (95% CI)**	**p**
	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)		
QFT positive											
	Overall (N=)			**X group (N=)**			**Y group (N=)**			**HR (95% CI)**	**p**
	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)	Events	Child-years	Incidence per 100 CY (95% CI)		
TST positive											

Table 2c. Exploratory combined endpoint of MTB infection, TB diagnosis, and death by study arm.

Outcome	X group (N=)			Y group (N=)			Effect Estimate		
	n	Person-time	Incidence rate (/100)	n	Person-time	Incidence rate (/100)	Hazard ratio	(95% CI)	*p*-value
MTB infection, TB disease or Death									

Table 2d. Proportion of exploratory outcomes due to MTB infection, TB diagnosis or Death (first event only) (Open report)

Outcome	Overall (N=)	
	n	%
MTB infection		
TB diagnosis		
Possible		
Probable		
Confirmed		
Death		

5.0 QUARTERLY SUMMARIES

Quarterly summaries will be circulated to study co-investigators, study monitors, and the DSMB. Open reports will include data aggregated over both arms (Figure 2, Table 1b). Closed reports with data separated by arm will only be made available to the study statistician and DSMB members. The summary will include a summary of adverse events and severe adverse events by study arm for the Closed report (Table 3a) in aggregate for the Open report (Table 3b). At the request of the DSMB, summaries will be sent to the DSMB by the study statistician. We anticipate DSMB meetings to occur at study initiation, 50% enrollment, 25% study endpoint ascertainment. ***At the guidance of the DSMB quarter summaries were reduced to approximately every 6 months.***

6.0 INTERIM ANALYSIS AND ANTICIPATED DSMB MEETING

Interim analyses for the risk of MTB infection and the exploratory endpoint will be performed when 25%, 50%, and 75% of expected enrolled children have reached 12 months. O'Brien-Fleming boundaries for benefit and harm will be used for interim monitoring and these boundaries will be provided by the study statistician in the closed reports. The DSMB will review the interim reports and assess the study in terms of operational aspects, safety, and effectiveness and will make recommendations regarding continuation or modifications of the study. Futility will not be a basis for stopping rules because of the trials' value in understanding epidemiologic and immunologic correlates of MTB infection in HEU infants. We anticipate DSMB meetings to occur at study initiation, 50% enrollment, and at 25%, 50%, 75% and 100% of study endpoint ascertainment. ***At the guidance of the DSMB the number of formal interim analyses of the primary endpoint were reduced to one formal DSMB meeting at approximately 50% enrollment, with administrative report reviews reduced to approximately Q 6 months.***

7.0 SAFETY MONITORING

Adverse and severe adverse events (SAEs) will be monitored by the DSMB. SAE summaries of severe adverse events (SAEs) will be sent to the DSMB members during DSMB review and individual-child SAE forms sent to the safety officer per request. Each SAE will be assigned the plausibility of relatedness to study drug which will be assigned by study PIs. The data will not be presented in these reports by intervention group unless requested by DSMB. These reports will be descriptive (no statistical analyses). At the DSMB meetings to review interim analyses the SAE data will be presented by arm for review and the DSMB will make recommendations regarding any imbalances in safety outcomes. ***At the guidance of the DSMB, TB was removed from the SAE reporting.***

Table 3a. Adverse event reporting by study arm shell table (Closed report)

	Overall (N=)		**X group (N=)**		**Y group (N=)**	
SAE	n	%	n	%	n	%
Total participants with SAEs*						
Total SAEs						
Death[a]						
Hepatic Failure						
HIV infection						
Hepatotoxicity*						
Peripheral neuropathy*						
Hospitalization[a,b,c]						
Pneumonia/URTI/flu						
Gastroenteritis						
Malaria						
Other[d]						

Table 3b. Severe Adverse event reporting overall shell table (Grade 3 or above) (Open report)

	Overall (N=)	
Characteristic	n	%
Total participants with SAEs*		
Total SAEs		
Death[a]		
Hepatic Failure		
HIV infection		
Hepatotoxicity*		
Peripheral neuropathy*		
Hospitalization[a,b,c]		
Pneumonia/URTI/flu		
Gastroenteritis		
Malaria		
Other[d]		

8.0 REFERENCES

1. Cotton MF, Schaaf HS, Lottering G, Weber HL, Coetzee J, Nachman S. Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.
2. Madhi SA, Nachman S, Violari A, Kim S, Cotton MF, Bobat R*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
3. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ*, et al.* The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:392-402.
4. Madhi SA, Nachman S, Violari A, Kim S, Cotton MF, Bobat R*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
5. Zar HJ, Cotton MF, Strauss S, Karpakis J, Hussey G, Schaaf HS*, et al.* Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007,**334**:136.
6. Cranmer LM, Kanyugo M, Jonnalagadda SR, Lohman-Payne B, Sorensen B, Maleche Obimbo E*, et al.* High prevalence of tuberculosis infection in HIV-1 exposed Kenyan infants. *Pediatr Infect Dis J* 2014,**33**:401-406.