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Infant TB Infection Prevention Study (iTIPS): a randomised trial protocol evaluating isoniazid to prevent *M. tuberculosis* infection in HIV-exposed uninfected children

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ABSTRACT

Introduction HIV-exposed uninfected (HEU) infants in tuberculosis (TB) endemic settings are at high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in the absence of known Mtb exposure. Because infancy is a time of rapid progression from primary infection to active TB disease, it is important to define when and how TB preventive interventions exert their effect in order to develop effective prevention strategies in this high-risk population.

Methods and analysis We designed a non-blinded randomised controlled trial to determine efficacy of isoniazid (INH) to prevent primary Mtb infection among HEU children. Target sample size is 300 (150 infants in each arm). Children are enrolled at 6 weeks of age from maternal and child health clinics in Kenya and are randomised to receive 12 months of daily INH ~10 mg/kg plus pyridoxine or no INH. The primary endpoint is Mtb infection, assessed by interferon-gamma release assay QuantiFERON-TB Gold Plus (QFT-Plus) or tuberculin skin test after 12 months post-enrolment. Secondary outcomes include severe adverse events, expanded Mtb infection definition using additional QFT-Plus supernatant markers and determining correlates of Mtb infection. Exploratory analyses include a combined outcome of TB infection, disease and mortality, and sensitivity analyses excluding infants with baseline TB-specific responses on flow cytometry.

Ethics and dissemination An external and independent Data and Safety Monitoring Board monitors adverse events. Results will be disseminated through peer-reviewed journals, presentations at local and international conferences to national and global policy-makers, the local community and participants.

Trial registration number NCT02613169; Pre-results.

Strengths and limitations of this study

- Most children born to mothers living with HIV are HIV-exposed and uninfected (HEU) but remain at high risk of tuberculosis (TB), making them an important population in which to study TB prevention.
- Current TB prevention guidelines do not recommend routine isoniazid (INH) preventive therapy (IPT) for children (including HEU) without a known TB contact, though recent data suggest the majority of transmission to children occurs outside the household due to community or unperceived household TB; therefore, a strength of our study is the enrolment of children without known TB exposure.
- Because of the high risk of progression to TB disease in infants, our strategy, which focuses on prevention of primary *Mycobacterium tuberculosis* (Mtb) infection as detected by a combined endpoint of interferon-gamma release assays and tuberculin skin test is novel, despite known limitations of both assays.
- Widespread implementation of IPT in adult people living with HIV (including peripartum women) could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed.
- Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, a randomised controlled trial design would provide important information regarding INH efficacy for primary prevention in this high-risk population.

INTRODUCTION

HIV-exposed uninfected (HEU) infants in tuberculosis (TB) endemic settings have a high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in absence of known Mtb exposure.[1–3] Because infancy is a time of rapid progression from primary infection to active TB,[4] it is important to know how TB preventive interventions exert their effects to build new strategies that adapt or extend approaches used in adults. Protecting HEU infants during this vulnerable period of immunodeficiency may provide long-term benefits.

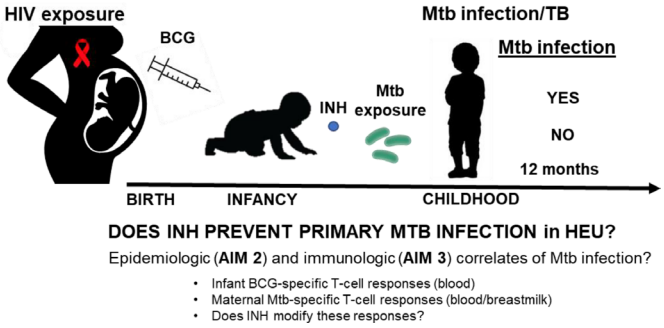

Figure 1 Study schema: aims of a randomised controlled trial to evaluate isoniazid (INH) to prevent *Mycobacterium tuberculosis* (Mtb) infection in HIV-exposed uninfected (HEU) infants.

Children have a higher rate of progression from Mtb infection to active TB disease than adults.[5 6] In young children who lack pre-existing adaptive immune responses, Mtb infection may progress rapidly to TB disease, and both innate and early adaptive immune responses likely influence susceptibility.[5 7–9] Virtually all childhood TB disease reflects primary disease, in contrast to adults where a significant portion of disease is due to reactivation of latent TB infection.[10] World Health Organization (WHO) recommends TB preventive therapy, including isoniazid (INH) preventive therapy (IPT), be provided to people living with HIV (PLHIV) >12 months of age to prevent TB.[11] Among children living with HIV (CLHIV), three randomised controlled trials (RCTs) yielded conflicting data regarding whether INH prevents TB disease and/or mortality.[2 12–14] Only one evaluated HEU infants and found no protective effect in decreasing a composite outcome of TB disease, Mtb infection (as measured by tuberculin skin test (TST)) or mortality.[2] While previous RCTs have focused on prevention of TB disease, there are scant data regarding INH impact on primary Mtb infection. Among 6-month-old HEU infants in Kenya, we found 10% had evidence of Mtb infection by interferon-gamma release assays (IGRAs), suggesting a potential 20% annual cumulative incidence of Mtb infection.[15] In a South African birth cohort including 22% HIV-infected mothers, TST test conversion incidence was 11.8 per 100 child-years, with the majority of conversions occurring before 1 year of age.[16] Among Ugandan children (median age 36 months), prevalence of Mtb infection (either TST or positive IGRA) was two-fold higher among HEU compared with HIV-unexposed children (HUU), with higher prevalence of TST positivity versus IGRA in both groups (HEU TST 27.2% vs IGRA 6.4%, HUU TST 20.6% vs 1.5%).[17] This suggests HEU infants have a substantial incidence of Mtb infection,[1 18] as well as low to modest concordance between different Mtb infection measures.

Study Design	Non-blinded randomised controlled trial
Intervention	Intervention: INH for 12 months Control group: No INH
Primary Outcomes	Aim 1: Mtb infection in HEU infants at 12 months post-enrolment as measured by IGRA (QFT-Plus) and/or TST 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 birthweight <2.5 kg
Target Enrolment	300 HEU infants and their HIV-infected mothers (150 each arm)
Sampling Framework	Consecutive enrolment of HEU infants and their HIV-infected mothers at MCH/PMTCT clinics in western Kenya

Figure 2 Overall study strategy. HEU, HIV-exposed uninfected; IGRA, interferon-gamma release assay; INH, isoniazid; MCH, maternal child health; Mtb, *Mycobacterium tuberculosis*; PMTCT, prevention of maternal to child transmission; TST, tuberculin skin test.

There is currently no gold standard for Mtb infection diagnosis.[19] While TST is recommended in children under 5 years of age,[20 21] false positivity due to BCG vaccination can occur.[22] Data regarding IGRA performance in young children are limited; however, a recent study of BCG-immunised infants in South Africa indicated high QuantiFERON interferon gamma (IFN-γ) conversion values were strongly associated with subsequent development of TB.[23] Because IGRAs offer increased specificity in the presence of recent BCG vaccine, it is plausible they could enhance the ability to measure the preventive effect of INH.[22] Cross-reactivity to non-TB mycobacteria may cause false positives in both IGRA and TST.[19] IGRA and TST agreement in children varies widely and appears affected by nutrition and HIV status, TB burden and BCG immunisation.[24–28] Recent American Thoracic Society (ATS)/Infectious Disease Society of America/Centers for Disease Control and Prevention guidelines recommend dual testing with IGRA and TST for groups who are both likely to be infected and at high risk of progression to TB disease as a strategy to increase diagnostic sensitivity.[21] Reduction of specificity with this strategy may be acceptable when consequences of missed Mtb infection (and therefore missed opportunity for treatment) outweigh risks of therapy-associated adverse events. Few longitudinal studies among young infants including HEUs with serial IGRA and TST testing exist.[23 29] A prospective infant HEU cohort using both IGRA and TST can provide an efficient approach to probe determinants of Mtb infection, more rapidly accruing endpoints (Mtb infection) than studies of TB disease. This study design can contribute unique insights regarding prevention strategies.

Kenyan guidelines mirror WHO and recommend IPT for all known TB-exposed children <5 years of age and all CLHIV >1 year of age regardless of TB exposure.[11 30] However, for children <5 years without known TB exposure (including HEU), and for CLHIV <1 year, IPT is not recommended.[11 30] These guidelines illustrate uncertainty regarding IPT in young children, following an RCT from South Africa/Botswana that failed to demonstrate IPT effectiveness in preventing TB disease among CLHIV and HEU <1 year of age without known TB

exposure.[2 12–14] It remains possible that among HEU children exposed to unperceived community or household TB, INH may prevent primary Mtb infection. Although data are conflicting, some adult studies have demonstrated IPT benefit in TST-negative or IGRA-negative adult PLHIV suggesting IPT may confer protection from Mtb infection.[31–34]

The primary goal of this study is to determine whether INH 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 (figure 1). This paper details the study protocol of an RCT evaluating efficacy of a 12-month course of INH to prevent Mtb infection as measured by IGRA and/or TST in HEU children enrolled at 6 weeks of age in western Kenya.

METHODS AND ANALYSIS

Study design

The infant TB Infection Prevention Study ('iTIPS') is a two-arm, non-blinded RCT comparing efficacy of a 12-month course of daily INH versus no INH to prevent Mtb infection among HEU Kenyan children enrolled at 6 weeks of age (figure 2). Eligible infants are randomised using a 1:1 allocation to INH versus no INH (figure 3).

Study sites

Kenya is one of 22 high TB burden countries with a generalised TB epidemic,[35] with an estimated TB prevalence of 426 per 100 000.[36] This study is conducted in collaborative research sites in western Kenya embedded in Ministry of Health (MOH) maternal child health (MCH) clinics. HIV-infected mothers are followed as part of the national prevention of maternal to child transmission (PMTCT) programme and currently receive Option B+ triple antiretroviral therapy (ART).[37] Per Kenyan guidelines, all PLHIV should be screened at routine HIV care visits using symptom-based TB screening and those with negative screens are evaluated for IPT.[11 35 37]

Recruitment processes and eligibility criteria

We recruit mothers living with HIV and their HIV-exposed infants from MCH/PMTCT sites. Infants 6 weeks (+4 weeks) of age are eligible for inclusion if they are born to HIV-infected mothers, with birth weight ≥2.5 kg, and not born premature (≥37 weeks' gestation). Infants

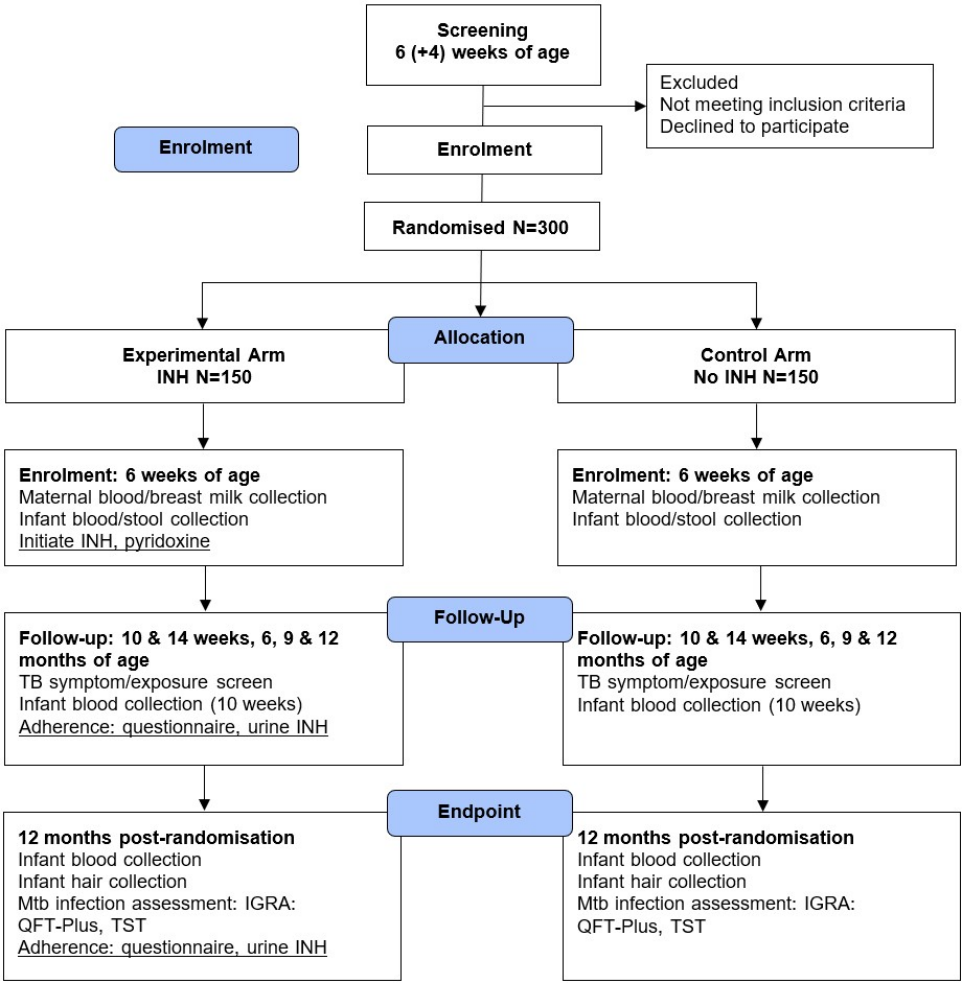

Figure 3 CONSORT diagram. IGRA, interferon-gamma release assay; INH, isoniazid; Mtb, *Mycobacterium tuberculosis*; TST, tuberculin skin test.

with known household TB exposure, including mothers with TB diagnosed in the past year, are ineligible. Infants enrolled in other TB prevention or TB vaccine studies are ineligible because these interventions might affect ascertainment of endpoints.

Randomisation

Site-stratified randomisation is used to allocate infants 1:1 to INH or no INH arms. Randomisation numbers were generated prior to study start using STATA V.14 "*ralloc*" command with resulting randomisation assignment by participant ID printed on cards and placed in opaque envelopes.

Blinding

The study is non-blinded to enable prompt clinical management for any potential drug-related adverse event. IGRAs are performed in the Kenya Medical Research Institute (KEMRI) Centers for Disease Control (CDC) laboratory, which is blinded to arm. The study team administers TST and is not blinded to TST result. Data monitoring by the study team is not disaggregated by study arm. The study biostatistician reviews data by arm during preparation of reports to the external Data and Safety Monitoring Board (DSMB). These data are reviewed during closed DSMB sessions, which excludes team members involved in study implementation.

Enrolment and study procedures

Enrolment

After informed consent is obtained by study staff, household locator information, medical identification number and cellphone contacts are obtained to facilitate tracing. On enrolment, standardised questionnaires regarding sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and history, and TB symptoms (for infant, mother and household members (by maternal report) using WHO symptom screen[38]) are administered (online supplementary table 1). Mothers with suspected TB are referred to the TB programme for further screening. If mothers are found to have TB on enrolment, their infants are ineligible for participation and are referred to receive INH per Kenya national guidelines.

Infants undergo physical examination measuring weight, height/length, mid-upper arm circumference and presence of BCG scar. Medical records are used to abstract data on infant birth weight, PMTCT prophylaxis, other medications, immunisations, and maternal ART regimen, viral load and CD4 cell counts.

Intervention

INH ~10 mg/kg (7–15 mg/kg) is administered once daily to infants in the INH arm for 12 months. Standardised weight-based dosing (by weight band using 100 mg scored tablets) is used, corresponding to Kenya and WHO recommendations.[30 39] Pyridoxine is provided to children randomised to INH to decrease peripheral neuropathy risk.[30 39] Caregivers are advised on how to pulverise INH and pyridoxine to be mixed with small quantities of breast milk, clean water or liquid co-trimoxazole to ensure full doses are given and for ease of administration to infants. Participants in the intervention arm are administered daily INH and pyridoxine by caregivers. Infants in the control arm do not receive INH or pyridoxine.

Participant follow-up

Follow-up visits occur at 10 weeks for infants enrolled at 6 weeks of age and at 14 weeks, and 6, 9 and 12 months of age for all participants coinciding with routine Kenya paediatric visit schedule. Follow-up visits include assessment of any TB diagnosis in the mother, infant or household member since the past visit, as well as any TB symptom in the mother, infant and household members (by maternal report). Infants in the no INH arm found to have a known TB contact during the study are referred for IPT per Kenyan guidelines. Infants and mothers found to have TB symptoms are referred to the MOH TB Programme for further evaluation. Questionnaires regarding caregiver barriers and facilitators to providing prophylactic medications (co-trimoxazole, antiretrovirals for PMTCT and INH (if in INH arm)) are administered at the 6 month of age visit. Endpoint ascertainment occurs at a study visit 12 months post-randomisation at approximately 14 months of age.

Sample collection

Infant blood for peripheral blood mononuclear cells (PBMCs) and plasma are collected at baseline and visit 2 (10–14 weeks of age). At 12 months post-randomisation, blood is collected for IGRA (QFT-Plus) and TST placed and read within 48–96 hours.[40 41] Infant rectal swabs are collected at enrolment for future gut microbiome studies. Maternal breast milk and blood for PBMC and plasma separation are collected on enrolment.

Study procedures specific to infants randomised to INH

Liver function tests (LFTs) are performed at enrolment and 1 month following INH initiation. Adherence is assessed by caregiver report at follow-up visits. Urine is collected at follow-up and study endpoint visits and tested using strips developed to detect INH metabolites.[42 43] Hair is collected at study endpoint for future assessment of INH levels as a more objective adherence measure over time.[44 45]

Safety considerations

IPT has been shown to be safe in prior RCTs and is administered routinely to TB-exposed infants.[2 12–14] Although routine LFT monitoring is not recommended during INH in children,[20] for this trial baseline LFTs are drawn at enrolment and 1 month after INH initiation. National Institutes of Health (NIH) Division of AIDS (DAIDS) Table for Grading the Severity of Paediatric Adverse Events is used to grade toxicities.[46] Infants with LFTs ≤grade 2 are allowed to initiate INH. Infants with baseline LFTs ≥grade 3 at baseline have LFTs monitored every 2 weeks and do not initiate INH until LFTs are ≤grade 2. Children are evaluated for peripheral neuropathy using a

truncated Denver Developmental test.[47] After INH initiation, if toxicity is suspected, study-administered drugs are immediately discontinued and in case of concern for hepatoxicity, LFTs are repeated.

An external and independent DSMB, including experts in paediatric TB, biostatistics and trial design, monitors severe adverse events (SAEs). Summaries of SAEs are given to DSMB members during scheduled meetings. Each SAE is assigned plausibility of relatedness to study drug by study investigators. 'Open' reports detailing cumulative overall SAEs are descriptive (no statistical analyses). 'Closed' reports of SAEs by study arm are reviewed and the DSMB makes recommendations regarding any imbalances in safety outcomes. O'Brien-Fleming boundaries for benefit and harm are used for interim monitoring, and these boundaries are provided by the study statistician in closed reports. The DSMB assesses operational aspects, safety and effectiveness, and makes recommendations regarding study continuation or modifications. Futility is not considered a basis for stopping rules because of the trial's value in understanding correlates of Mtb infection in HEU infants.

Discontinuation, withdrawal or allocation modification

Participants may withdraw from the study at any point. Study investigators may withdraw a participant on a case-by-case basis if the intervention or study involvement poses a risk to the participant. No modification of allocation will be made. Infants who receive at least one dose of study drug will be included in per-protocol analyses. Caregivers of infants who discontinue INH are encouraged to continue study follow-up and endpoint ascertainment.

Data collection and management

Study staff use tablets to collect de-identified data using secure password-protected Research Electronic Data Capture mobile software (REDCap; Vanderbilt University, Nashville, Tennessee, USA).[48] Data are uploaded daily from tablets to the web-based REDCap database. Study investigators will have access to the finalised dataset.

Patient and public involvement

Patients were not directly involved in the development of the research question, design of the study or recruitment. We assess the burden of the trial intervention for participants by gathering data on adverse events, tolerability of INH, and assessment of caregiver barriers and facilitators to providing prophylactic medications to HEU children via questionnaire. Overall study results will be shared with the clinical facilities and presented to local stakeholders including MOH county and national representatives.

Outcome measures

The primary outcome is Mtb infection by QFT-Plus assay or TST 12 months after enrolment. Similar to QuantiFERON-TB Gold (QFT) IGRA, QFT-Plus measures IFN-γ released by primarily CD4+ T helper lymphocytes after TB-specific antigen (ESAT-6 and CFP-10) stimulation. In addition, QFT-Plus measures IFN-γ released by CD8+ cytotoxic T lymphocytes, after stimulation with the same antigens, which may have increased sensitivity in children, and in populations with lower CD4 counts including PLHIV.[49 50] Responses of ≥0.35 IU/mL to TB antigens above the Nil response in either the primarily CD4+ (TB1) or CD8+ response (TB2) (with Nil <8 IU/mL and a positive mitogen control) are considered positive per manufacturer recommendations.[49] A TST of ≥10 mm is considered positive.[20]

Secondary outcomes include severe adverse events (grade ≥3 per DAIDS Grading Severity of Paediatric Adverse Experiences),[46] use of IFN-γ-independent immune markers in QFT-Plus supernatants to indicate Mtb infection,[51–55] and epidemiological and immunological correlates of Mtb infection. Exploratory outcomes include combined endpoint of Mtb infection, TB diagnosis and/or death, as well as sensitivity analyses of the primary outcome of Mtb infection after excluding infants with evidence of immune responses to ESAT-6 or CFP-10 at enrolment in flow cytometric analyses.

Sample size and power analysis

Assuming an alpha of 0.05, power of 0.80, using a two-sided test and a 1:1 allocation ratio, with 125 infants in each arm, we have power to detect at least a 65% decrease in Mtb infection in INH arm versus control if cumulative incidence of Mtb infection in the control arm at 12 months is 0.20, or to detect 70%–80% or higher (HR 0.3–0.2) decrease if cumulative incidence of Mtb infection in the control arm is 0.15 or 0.10 (online supplementary table 2). To account for loss to follow-up, non-adherence and INH resistance, we increased sample size by 20%, with goal enrolment of 300 infants (150 per arm). Baseline characteristics will be compared between randomisation arms to assess randomisation adequacy.

Statistical methods and analysis

Primary outcome

Modified intention-to-treat: We will use a modified intention-to-treat approach, including all participants who underwent randomisation irrespective of receiving trial medication with at least one measure of Mtb infection (QFT-Plus or TST), excluding children found to be HIV DNA positive during the study. We will compare the proportion of infants with Mtb infection (either QFT or TST positive) at 12 months between INH and no INH arms using a χ^2 test and estimate relative risk with 95% CIs using a generalised linear model with log link and Poisson family. We will compare cumulative incidence of Mtb infection by arm using a Cox proportional hazard regressions model.

Per protocol: We will evaluate our primary outcome by a per-protocol analysis, considering only HEU infants who took at least one dose as taking INH versus 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.

Secondary outcome

Safety and expanded Mtb infection outcomes: For secondary outcomes, we will compare proportions of participants by arm using either χ^2 or Fisher's exact tests as appropriate for ≥grade 3 serious adverse events. 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.

Epidemiological and immune correlates of Mtb infection will be assessed using nested case–control studies incorporating all Mtb infections from both arms then conducting stratified analyses in each trial arm to evaluate potential cofactors modified by INH.

Exploratory outcome

We will compare a composite endpoint of Mtb infection, TB diagnosis and/or death between randomisation groups using a χ^2 test. Baseline assays may detect evidence of Mtb infection. We will conduct additional exploratory analyses, incorporating data from baseline assays[56] (using flow cytometry of cryopreserved PBMCs) to exclude infants with evidence of Mtb-specific immune responses to ESAT-6 or CFP-10 at enrolment also using a χ^2 test.

ETHICS AND DISSEMINATION

Informed consent is obtained from caregivers. Any protocol changes will be approved by relevant ethical review boards. The protocol is available online (http://depts.washington.edu/gwach/wp-content/uploads/2012/10/iTIPS_Protocol_v1.8_01Nov2019.pdf).

We will share trial results at study sites, and with regional and national policy-makers. We plan on submitting final results as a peer-reviewed manuscript and will use International Committee of Medical Journal Editors authorship criteria. Study investigators will collaborate in writing final study results.

HEU children are at increased risk for Mtb infection and TB disease. IPT is not routinely provided to HEU infants in Kenya without evidence of exposure to a known TB case. There is mixed evidence regarding IPT effectiveness to prevent TB disease in infants <1 year. Given potential benefits of IPT to prevent Mtb infection, heightened risk for Mtb infection in this population and safety of intervention, there is equipoise for randomisation.

Trial status

Trial recruitment and enrolment began 15 August 2016. Participant follow-up is anticipated to complete October 2019, with laboratory analyses of the QFT-Plus endpoint anticipated to be completed in December 2019.

DISCUSSION

INH has proven benefit to treat latent TB infection and prevent active TB disease in HIV-infected and HIV-uninfected populations.[57–61] Data from adult studies in Botswana, South Africa and Ivory Coast indirectly suggest IPT may prevent Mtb infection; TST-negative adult PLHIV who received IPT were protected from active TB, suggesting IPT may both prevent Mtb infection and progression to TB disease.[31–34] IPT has had variable protective efficacy to prevent TB disease and mortality in CLHIV.[2 12–14] An RCT in South Africa in the pre-ART era randomised CLHIV ≥8 weeks of age to INH versus placebo independent of reported TB exposure and found INH prevented TB disease by 70% and decreased mortality by 54%, leading to early trial discontinuation.[12] In the observational extension of the trial, combination IPT and ART further decreased TB risk by 11%.[62] However, in a pilot study of CLHIV on ART (median age 35 months) not powered for efficacy, IPT did not exert a significant protective effect on active TB (1.5 vs 2.9 TB cases per 100 person-years, incidence rate ratio 0.51 (95% CI 0.15 to 1.75)).[13] Similarly, an RCT of INH given for 96 weeks in HIV-infected and HEU infants enrolled at 91–120 days of life in South Africa and Botswana without reported TB exposure did not prevent TB disease in either group.[2] Furthermore, among HEU, INH did not prevent Mtb infection as measured by a single TST at week 96. In summary, IPT is effective in adults and variably effective for preventing TB disease in HIV-infected and HEU infants, and no trial to date has been designed specifically to evaluate efficacy of IPT to prevent Mtb infection in either adults or children, including both IGRA and TST as an endpoint to maximise sensitivity to identify Mtb infection.

Study limitations

Enrolment sites are limited to two counties in western Kenya and may not be generalisable to other settings. This area was chosen due to high HIV/TB burden, as well as longstanding collaborations with study investigators in enrolling women and children from MCH/PMTCT clinics. With non-blinded trials, there are concerns about differential reporting and clinical management. However, one of the composite endpoint components (IGRA) is assessed in the KEMRI CDC laboratory, which is blinded to participant INH status. This endpoint is robust and not influenced by unblinded trial design. We have estimated a substantive INH effect (65% decrease), consistent with TB prevention literature for reduction of TB disease among TST-positive adult PLHIV,[60] but undefined for Mtb infection risk. A larger sample size may be useful if Mtb infection prevalence is lower than anticipated or if INH is less effective in prevention of Mtb infection. We have extended post-trial observational follow-up to 24 months of age to assess longer-term Mtb infection incidence. This extended follow-up will allow us to better understand timing of Mtb infection acquisition; however, results will not be included in the primary trial results because the extended observational period will not include receipt of IPT.

There remains a lack of a gold standard to diagnose Mtb infection[19]; both TST and IGRA are indirect measures of

Mtb infection requiring both infection with Mtb and a functioning immune system to mount a positive response. We have incorporated both tests within our composite primary outcome. TST at 12 months may be positive due to BCG exposure at birth rather than Mtb infection. Age at immunisation and TST testing timing after BCG administration appears to affect TST reactivity, with younger age at BCG immunisation associated with shorter duration of TST reactivity than in adults. In a meta-analysis of 24 studies with >240 000 participants, among those who were BCG-vaccinated as infants, <1% were TST positive after 10 years post-BCG administration, compared with 21% of participants vaccinated after their first birthday who remained TST positive after 10 years post-BCG.[63] Similarly, in a recent long-term follow-up study of a BCG versus placebo trial among Native Americans/Alaskan Natives, BCG administered after 1 year of age was associated with increased incidence of TST reactivity extending up to 55 years after vaccination.[64] Importantly, there are scant data on TST reactivity among BCG-immunised infants TST tested during the first year of life. Among 250 Navajo infants immunised with BCG as newborns, 31% had TST ≥10 mm at 3 months which reduced to zero at 9 months of age, suggesting rapid waning of BCG-associated TST responses in children receiving BCG at birth.[65] Therefore, it appears that TST testing at approximately 1 year of age among children immunised with BCG at birth is more likely to represent Mtb infection, as opposed to BCG-induced reactivity. Non-tuberculosis mycobacteria (NTM) can lead to false positives for both IGRA and TST.[19] Prevalence of NTM disease in Kenya is unknown, but in a recent study evaluating 2900 infants for TB incidence in Kenya, 2.6% of infants evaluated for TB had NTMs isolated, though none met ATS criteria for NTM disease.[66] Importantly, the study evaluated detection of mycobacteria rather than detection of subclinical NTM infection, and there are currently no standard measures for NTM infection. Our study does not include qualitative work to investigate issues of adherence, though it does include closed-ended questions regarding caregiver barriers and facilitators to providing prophylactic medications to HEU children.

Kenya endorsed routine IPT for PLHIV in 2014 national guidelines,[37] and counties in which this study is located have had a rapid expansion of IPT as part of routine HIV care. We have described high IPT use in peripartum women.[67] Widespread IPT implementation in adult PLHIV could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed. Maternal IPT use is not an exclusion criterion. Infant INH drug exposure through breast milk is very low[68] and unlikely to exert a direct protective effect in the control arm.

Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, an RCT design could provide important information regarding INH efficacy for primary prevention in this high-risk population.

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Contributors GJ-S, BAR, JK and SML designed the randomised clinical trial. SML, GJ-S, BAR, TRH, LMC, JK, DM, AJW and EM-O developed the study protocol. GJ-S is the principal investigator and protocol chair and TRH is the immunology principal investigator. JK is the protocol co-chair and country principal investigator. EM-O is the Pediatric Clinical TB lead. GJ-S, BAR and SML are responsible for the statistical design of the trial and data analysis of the primary outcomes. SML is the project director and drafted the statistical analysis plan overseen by BAR, the study biostatistician. SML, DM, AJW, JK and JNE participated in trial implementation and manuscript preparation. TRH designed the immunological studies and oversees the immunological work related to the trial. SML wrote the first draft of the manuscript. All authors critically revised, read and approved the final manuscript.

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