ISONIAZID PLUS COMBINED ANTIRETROVIRAL THERAPY TO PREVENT TUBERCULOSIS IN HIV-INFECTED PERSONS: A PRAGMATIC RCT

ART-IPT study: University of Cape Town

Investigator(s)
Prof. Gary Maartens (Principal Investigator)
Dr Eric Goemaere
Dr Robert J Wilkinson
Dr Gilles van Cutsem
Dr Andrew Boulle
Dr Katherine Hildebrand
Dr Shaheed Mathee
Dr Molebogeng X Rangaka (Lead Investigator and Project Coordinator)

Collaborators
Dr J Glynn (Infectious Diseases Epidemiology Unit, London School of Hygiene and Tropical Medicine)
Dr K Fielding (Infectious Diseases Epidemiology Unit, London School of Hygiene and Tropical Medicine)

Main centre(s)
Ubuntu Clinic, Site B Day Hospital, Khayelitsha, Cape Town, Western Cape

Contact details
Address: Ubuntu Clinic
Site B Khayelitsha Day Hospital
Lwandle Road
Khayelitsha
Cape Town
South Africa
Phone/fax) +27 21 361 0359

Address: University of Cape Town Office
Room S3.03 Level 3
Wolfson Pavillion
Institute of Infectious Diseases and Molecular Medicine
Werner & Beit Building
UCT School of Health Sciences
Anzio Road
Observatory
Cape Town
South Africa
Phone: +27 21 446 6389 Fax +27 21 446 6796

1 Summary

The incidence of Tuberculosis (TB) in poor settlements around Cape Town continues to rise despite combination antiretroviral therapy (ART) roll-out and directly observed therapy for TB. In Khayelitsha district, where this project is conducted, TB incidence exceeds 1600/100000 per annum and the HIV prevalence reported in ante-natal clinics is 33%. Over 70% of adults presenting with active TB at our Khayelitsha clinic are co-infected with HIV and a third of all patients starting ART have active TB. Although ART has been shown to reduce the overall risk of TB by 59-80%, this risk still far exceeds the risk in the HIV uninfected people. In the Khayelitsha ART cohort, the risk of developing TB whilst on ART is ~12 per 100 p-y. In the nearby community of Gugulethu, there is a 14% p.a risk of active TB with at least half of the cases occurring within the first 3 months on ART. In a region where M.tb-antigen-specific T-cell based detection of latent TB infection in HIV infected adults is at least 80%, there is a real concern that TB will likely undo the benefit of ART. Additional measures are therefore required to reduce the risk of TB in those already receiving or starting ART. Isoniazid preventive therapy (IPT) represents an option but there is insufficient evidence to determine whether IPT can further (and safely) reduce the risk of TB in the ART era. In a pragmatic RCT, we propose to evaluate whether IPT can reduce the risk of active TB in patients receiving ART. A total **minimum sample size of 1368** is required for the study to detect a 35% reduction in the hazard rates for tuberculosis in the intervention group compared to the control group at a power of 80% and a Type I error of 0.05. Development of TB will be the primary endpoint.

Table of Contents

2 Background

Defective cellular immunity in persons infected with HIV-1 and *M. tuberculosis (M.tb)* results in greater susceptibility to develop active TB with an estimated incidence risk of 7%-10% per annum.(1, 2) The role of isoniazid preventive therapy (IPT) in decreasing the risk of TB in HIV infected people who are tuberculin skin test (TST) positive is well documented in Cochrane reviews. The identification and subsequent administration of IPT to this group remains a high priority for TB control programs. There is, however, very little evidence base for the potential role of IPT in reducing the risk of TB in those receiving or commencing ART.

HIV associated TB in South Africa is increasing despite directly observed therapy for TB and ART roll out.(3) 76% of TB at our Khayelitsha community HIV/TB clinic is associated with HIV.(4) and a third of patients starting ART have active TB. This increasingly affects the capacity of TB control programs to cope with the growing dual epidemics. Combined ART reduces the risk of TB and in Cape Town, this reduction is between 59% and 80%. (5) (6) However the risk of acquiring TB in those receiving ART still greatly exceeds the risk of HIV uninfected people by 10 to 1.(7) TB incidence rates on ART range between 10%-14% per annum in the Khayelitsha community clinic cohort, even in those on ART for over two years. In a nearby community, 14% of patients on ART develop TB within the first year and 52% of these cases occur within the first 3 months. Paradoxically, the gain in life expectancy due to ART may lead to a larger number of people in the community who develop and potentially transmit TB.(8) In the absence of an effective and safe vaccine against TB in HIV infected people, IPT presents a promising preventive option.

However, there has not been any prospective randomized evaluation of the potential of IPT to further reduce the risk of TB in HIV infected people receiving or starting ART. Furthermore, the safety of IPT co-administered with ART is unknown. In addition to combined drug toxicities, concerns include increased pill burden, which may result in poor adherence of both drugs that may lead to poor ART outcomes, and finally, the potential for INH mono-resistance.

2.1 Observational studies of IPT and ART

A StopTB policy statement on IPT for HIV infected people issued at the 2007 IUATLD has made a strong recommendation for co-prescribing IPT and ART. They have stated that co-prescribing IPT and ART ‘works’ and is ‘safe’. This recommendation, however, is based on data from a large retrospective folder review (N=6391) conducted in Rio, Brazil that evaluated the impact of implementing IPT in HIV clinics. This showed that compared to patients who received neither IPT nor ART, patients who had received ART and IPT had the lowest TB incidence 0.80/100 PY (95% CI 0.38-1.47), adjusted relative hazard 0.24; P < 0.001).(9) However, patients did not necessarily receive both ART and IPT at the same time (*pers. comm*. Richard Chaisson). Safety data from this trial has not been published.

Using the same methodology, the same authors reviewed data from two clinical cohorts of HIV-infected patients in South Africa. They similarly evaluated whether receipt of IPT and/or ART was associated with the risk of TB amongst a combined cohort of 2778 HIV-infected patients from various clinical sites. They showed that compared to patients who received neither IPT nor ART, patients who received ART

after IPT had a 89% reduced adjusted hazard rate (HR=0.11; 95%CI 0.02–0.78). The observational study design does not allow for an assessment of the association between ART *after* IPT and the risk of subsequent TB without confounders. Similarly, very little can be deduced about the association between ART *plus* IPT and the subsequent development of TB. This is yet to be answered definitively.

2.2 Combined Toxicities

INH for six months, recommended by the WHO, is the most widely implemented IPT in Africa.(10, 11)'(12). The current American Thoracic Society/Centre for Disease Control's recommended IPT duration in HIV infected persons is 9-12mo.(13) Shorter regimens with rifamycins have been found to be as efficacious as INH. (1, 14) However, Cochrane reviews report that the likelihood of treatment discontinuation due to adverse effects is higher for combination therapies than for INH monotherapy. For example, a Rifampicin-pyrazinamide combination is significantly associated with severe hepatotoxicity more often than INH or curative chemotherapy for TB.(15) Furthermore, Rifampicin is a potent inducer of cytochrome P450 enzymes and is associated with lower levels of many key antiretroviral drugs (notably protease inhibitors & non-nucleoside reverse transcriptase inhibitors).

Possible combined ART/IPT toxicities include increased peripheral neuropathy and hepatitis. Peripheral neuropathy is by far the most commonly reported adverse event from stavudine whilst serious hepatotoxicity is not uncommon with Nevirapine. Both Nevirapine and stavudine are included in the current first-line ART regimen in South Africa). There is a paucity of information regarding combined toxicities of ART regimens containing at least one of those drugs. Furthermore isoniazid inhibits the effects of the cytochrome P450 isoenzymes CYP3A4 which metabolises nevirapine and lopinavir/ritonavir. Inhibition of CYP3A4 will result in increased concentrations of these antiretrovirals, increasing the risks of toxicity. Reported studies of combined toxicities of INH co-prescribed with nevirapine and/stavudine have been small, retrospective and non-randomized.(16) A large controlled prospective study would help determine whether these combined regimens could be tolerated in HIV infected people on ART.

3 Research hypothesis

Isoniazid can further reduce the risk of tuberculosis in HIV infected people receiving combination antiretroviral therapy and can be safely co-prescribed with ART.

4 Aims

1. To evaluate the role of isoniazid preventive therapy in reducing the risk of active tuberculosis in patients receiving ART.
2. To determine the adverse effects of co-prescribing IPT and ART with respect to:
    a) drug toxicity
    b) adherence of ART
    c) the effect on ART outcomes
    c) isoniazid mono-resistance
    d) mortality

5 Primary and secondary endpoints

5.1 Primary

Effectiveness:

Development of TB

5.2 Secondary

Safety:

1. Drug toxicity (Adverse events ≥ Grade III and unexpected serious adverse events) in the intervention arm compared to the control arm
2. Worsening adherence to ART and the study drug as indicated by pharmacy refills in i) the control vs intervention arms, and ii) the study cohort compared with the general clinic cohort
3. Worsening ART outcomes (virological and immunological failure) in i) the control vs intervention arms, and ii) the study cohort compared with the general clinic cohort Any INH resistance in microbiologically confirmed incident TB cases in the intervention arm compared with the control arm.
4. All cause mortality, intervention arm compared with the control arm

6 Outcome measures

The hazard rate of TB in each study arm will be described as well as the hazard rate ratio. The rates of developing various serious adverse events during the intervention period will also be described.

7 Study Design

This is a stratified 1:1 individually randomized double-blinded placebo controlled trial of IPT in HIV infected persons on ART. Randomization is stratified by ART status at baseline: starting vs established on ART. Particiapants are given 12 months of selfadministered study drug. The follow-up period post intervention will be 24 months (that is, a total risk-period of 36 months: intervention plus follow-up).

This is a pragmatic trial that i) answers an urgent operational question for this clinic, ii) aims to demonstrate the effectiveness of the combined therapies and explore the feasibility of implementing a TB prevention program in the context of a busy integrated ART/TB Clinic in Khayelitsha, South Africa.

A major condition to conducting this trial at the Ubuntu clinic was that it should be closely linked with service provision and should be used to improve research capacity as well as service delivery and care at the clinic.

The trial is registered at www.clinicaltrials.gov (NCT00463086).

8 Description of the intervention

The intervention group self-administers a daily oral dose of isoniazid (INH) administered as 100 mg tablets at a dose of either 200 mg/d (for participant's weighing <50 kg) or 300mg/d (for participant's weighing ≥50 kg) and 25mg of pyridoxine Hydrochloride for 12months (treatment can be completed over a 15-month period to allow for a 3-month grace period added for those who interrupt treatment for whatever reason which may include TB investigations and missed appointments). The control group receives matching placebo INH tablets plus pyridoxine. The patients and researchers are blinded to specific drug allocations.

9 Project Plan and milestones

A detailed project work plan is provided in Appendix A.

Study start date: 1 November 2007
First patient enrolled: 31 January 2008
Anticipated study termination: January 2012

10 Study Setting

Khayelitsha township, with a population of ~500,000, is the largest of the urban settlements in Cape Town. The community is largely comprised of socially deprived migrants from rural areas residing in a mixture of formal and informal dwellings. Reasonably well-functioning primary care facilities are in place. Tuberculosis services in Khayelitsha have reported a steady increase in annual case notifications, reaching over 1600/100 000 by the end of 2007. 76% of adults starting TB treatment at Ubuntu Clinic are co-infected with HIV. The prevalence of HIV in women attending antenatal care is 33%. In 2000, Médecins Sans Frontières (MSF) with the support of the local health structures initiated the establishment of the Ubuntu ART Clinic, which was later integrated with TB services. Studies from this well-described cohort have contributed to greater understanding of ART outcomes in resource-limited settings. (17, 18) (19) (20) (21). This cohort continues to inform policy around ART roll-out and provision through the joint collaborative cohort study group, ART-LINC. The integration of HIV voluntary counselling and testing and antiretroviral services with TB programs enhances access to TB prevention and treatment. By the end of 2008, more than 10000 patients had been enrolled into care and over 7000 had started ART in Khayelitsha, ~3500 at the Ubuntu Clinic alone.

11 Inclusion and exclusion criteria

Male and female attendees (age ≥18yo) of the Ubuntu ART Clinic are eligible to participate. Participants are recruited amongst existing clinic attendees already on ART as well as from new patients about to commence ART. The following are exclusions to participation: 1) Evidence of active TB or suspicion of active TB as determined by a symptoms screening algorithm. 2) Current TB chemotherapy (TB treatment completed in the preceding 30 days will not be an exclusion) 3) Current or previous treatment of latent TB infection since HIV infection (any duration) 4) Current treatment with fluoroquinolones or other antibiotics with significant anti-tuberculous activity currently being used to treat TB in South Africa 5) Past reaction/intolerance to INH. 6) Acute hepatitis or existing Grade III-IV peripheral neuropathy. 7) Pregnancy or < 6weeks post-partum period (Due to increased risk of hepatotoxicity). 8) Grade III or higher baseline abnormal liver function. (Note: toxicity grades are all according to AIDS Clinical Trials Group (ACTG) toxicity tables for persons on ART).

12 The randomization process

For this pragmatic trial, we needed a method that is easy to implement at the clinic level whilst also ensuring that the patient and doctor remain blinded to study allocations. The randomization process flow-chart is available in Appendix B. Randomization is stratified according to ART status. This is simply defined as new on ART (not yet on treatment when screened) and established on ART (already on treatment when screened). The process described below applies for each stratum. An independent statistician randomly allocated isoniazid or placebo to 3 of 6 groups each, from 1 - 6. That is, three of the groups are placebo and the rest INH. Groups 1-6 were randomly assigned to a list of sequential numbers up to a 1000 for each stratum by using simple computer software. No formal blocking techniques were used, however, the statistician did ascertain whether the study groups 1- 6 were represented in similar frequencies within a patient stratum and that the groups 1-6

were also similar between strata. The randomisation key is stored on CD and is kept by the independent statistician. Two copies have been made: one copy is kept by an independent Pharmacist and a second copy by the departmental secretary (Pharmacology) and kept under lock and key.

The study drugs (INH and placebo) were ordered directly from the supplier and delivered to our independent Pharmacist at the University of Cape Town. The independent Pharmacist pre-packages a month's supply of INH or placebo in individual pill packets marked group 1- 6 as per randomization key. The pill packets are transported to Ubuntu Clinic pharmacy where they are stored according to their groups (as opposed to, by individual patient numbers). Labeling the pill packets with the drug group instead of individual enrolment numbers allows for easy storage of the drugs at Ubuntu Clinic where pharmacy space is limited. Only the study statistician and the independent pharmacist at UCT know which study drugs (INH or placebo) groups 1- 6 correspond to.

Two enrolment logs were created, one for new cases and the other for existing cases on ART. Sequential enrolment numbers with their corresponding drug groups 1- 6 were printed directly onto the enrolment logs. The logs display the following information: study enrolment number, clinic folder number, patient's name, enrolment date, the corresponding allocation group, the signature of the pharmacist/pharmacist assistant randomizing the patient, a confirmatory signature by pharmacy staff and the date and reason of study drug termination. These logs are kept at the Ubuntu Clinic Pharmacy under lock and key. The Pharmacy controls the allocation of study groups and therefore maintains study blinding.

On the day of enrolment, two clinicians (Nurse or Doctor) confirm study eligibility, verify participant consent and sequentially enter patients in an enrolment register that is controlled by the Study Coordinator. The enrolment numbers on the register correspond to the study numbers printed on the enrolment logs that are kept at the pharmacy. The study drug (INH/placebo) is prescribed according to the patient's weight and noted on a prescription chart. The patient then takes the prescription chart to the pharmacy.

At the pharmacy, patient details are sequentially entered in the enrolment log and the group allocation retrieved. The pill packet displaying the correct drug group for that particular study number is then dispensed to the patient together with a month's supply of pyridoxine. A second, authorised, pharmacy personnel has to confirm that the details on the pill packet to be dispensed are correct for a given study number before signing the pharmacy enrolment log. A generic label is then placed on the pill packet and any reference to the study group removed. These generic labels display the following info: the name of the study, the study number, study drug expiration date and the name of the dispensing clinic and instructions on how to take the drug. Once the confirmatory signature is placed on the pharmacy enrolment log, the drugs are then handed over to the patient.

The Study Coordinator and Pharmacist have the exact same information on the overall numbers and details of patients enrolled per day bar the group allocations. This information is reconciled on a weekly basis.

13 Participant Recruitment and Assessment of eligibility

Participants are recruited amongst **existing** clinic attendees already on ART as well as from **new** patients about to commence ART. Study Counsellors and Nurse Clinicians are responsible for all recruitment. The recruitment process for new ART clinic attendees as well as for those patients already receiving ART is described below.

13.1 New ART clinic attendees

Following mass education in the waiting room on IPT and TB, patients are invited to attend small group discussions. Patients referred to the clinic for ART initiation are further counselled on IPT and ART during pre-ART counselling sessions. Patients have to attend three preparatory counselling sessions 1 to two weeks apart before ART is initiated. At these sessions, information on ART and preventive treatment including IPT and co-trimoxazole is given to patients as a package and the role of IPT in HIV put into context. Patients that meet the trial's inclusion criteria are then invited to participate in the study and consent procedures followed prior to conducting any screening procedures. The consent process includes: 1. provision of information regarding the study objectives, methods, risks and benefits to participation and patient responsibilities. 2. Obtaining a signature from the patient as proof of written consent, those who cannot write, a thumb-print is obtained.

13.2 Patients already established on ART

Patients that are established on ART are assessed sequentially as they attend the clinic for scheduled appointments. They are counselled on IPT and TB at group discussions, invited to participate and consented for the study. Baseline clinical assessments are the same as for new clinic attendees not on ART. These are described in the next section.

14 Clinical and Laboratory Assessments

Baseline screening

Eligibility for the trial is determined via clinical and laboratory assessments. Study clinicians (Nurse/Doctor) are responsible for conducting these assessments. Clinical assessments include screening for symptoms and signs of active TB, excluding clinical hepatitis and grade III or worse peripheral neuropathy. Screening for TB is covered in detail below (also see Appendix C). Laboratory assessments include determination of a baseline ALT to exclude grade III or worse transaminitis (ALT ≥ 200). All assessments are conducted according to standard protocols adapted from those currently in use at the Ubuntu clinic. Assessment outcomes are recorded on the general clinic case report form. No separate clinical case report forms have been developed for the trial.

Screening for TB prior to study-drug initiation

Our screening tool consists of 2 components: Clinical screening and TB smear and culture. All components are assessed for each patient at the baseline visit. Case definitions for prevalent TB are summarised in Table 1:

Clinical screening

The clinical screening tool includes a combination of symptoms and signs. Patients with **one or more symptoms or signs** will be considered to be **TB suspects.** A standard check-list, printed on the clinic case report form, is used by trained staff to assess patients at clinical visits, and it includes the following:

a. Cough ≥ 2 weeks?

b. Drenching night sweats in the past two weeks?

c. Loss of weight ≥1.5kg in a month? (patients will be weighed on calibrated electronic scales and according to a standard operating procedure)

d. Fever ≥ 38 degrees Celsius ≥ 2 weeks?

e. Significant lymphadenopathy >2cm?

This clinical algorithm complies with various South African guidelines including the TB Program and the National IPT guidelines for HIV infected persons. It also draws on results by Mohammed and colleagues who found that an algorithm that includes two or more symptoms of weight loss ≥2.5% in 4 weeks, fever ≥ 2 weeks, cough and night sweats ≥ 2 weeks had a sensitivity of 100% and specificity of 88% to rule out active TB in HIV infected people with advanced disease (WHO Stage III/IV) and that chest radiography added very little information.

Lymphadenopathy > 2cm is the only variable that has not been validated, however, it has been suggested by the WHO TB/HIV collaborative as a clinical sign that may assist the diagnosis of tuberculous lymphadenitis and has, therefore, been included in this algorithm.

Sputum for auramine staining and mycobacterial culture

Patients submit a sputum specimen; produced spontaneously or induced following ultrasonic nebulisation with hypertonic saline. Specimens are sent to the South African National Health Laboratory Service at Groote Schuur Hosptial for processing (Bactec MGIT and LJ).

Patients that recently completed treatment for active TB are not excluded. They are assessed for study eligibility, screened as per protocol then commenced on the study drug if eligible. Patients that are TB suspects at the initial screening visit and therefore ‘fail’ the baseline assessment are investigated for TB till a final diagnosis is made (TB or not TB) and then referred appropriately. If TB is not confirmed, those patients are re-screened after a 4-week waiting period. Patients that are initially

screened but delay starting the study drug till a 3-month grace period has lapsed are also re-screened for TB (repeat cultures etc).

Table 1 Summarising TB Case Definitions used for prevalent active TB (TB detected during screening)

Definite TB	• If one or both cultures are positive for M.tb with or without compatible clinical or radiological features.
Probable TB	• If smear is positive for acid-fast bacilli (AFB) plus suggestive clinical features or radiological findings at baseline or during subsequent follow-up.
Possible TB	• If compatible clinical features plus suggestive radiological findings or failure to respond to antibiotics. • If radiological abnormality noted at baseline in the presence of no other sign or symptom worsens on repeat radiography up to 2-8 weeks, assessed at two weekly intervals after the first assessment plus patient subsequently develops compatible clinical features and a specialist physician/radiologist suggests features are consistent with current active TB. • Response to anti-TB treatment by 2 months
Not TB	• If all culture results are negative, irrespective of baseline symptoms

Note:
**Compatible clinical features include one or more of the symptoms or signs printed on the clinic case report form with or without any of the following: haemoptysis, shortness of breath, pleuritic chest pain, loss of appetite, extreme fatigue or lethargy, pallor or anaemia.*
***Compatible radiological features. Refers to films taken at baseline or during follow-up, up to 8 weeks since first presentation or until culture results are available*
****Response to antibiotics. Refers to a clinical and/or radiological response assessed two to eight weeks post first presentation.*
***** The 2007 WHO diagnostic algorithm for extra-pulmonary TB will be followed.*

15 Study enrolment

At the enrolment visit, study clinicians check patients' understanding of study procedures and confirm their eligibility to continue in the study. Screening data is examined for completeness and for abnormal results (specifically, raised ALT and any positive TB culture results) before a patient is assigned an enrolment number and the study drug prescribed. At that visit, the symptoms and signs screen for TB (already described) are revisited and questions to exclude clinical hepatitis and peripheral neuropathy asked before the study drug is prescribed.

INH or placebo is commenced within a month of starting ART in those newly initiating ART group. Screening for active TB does not delay ART initiation. The study team does not make any changes to ART regimens prior to prescribing the study drug to patients who are established on ART (See Table 2 for ART regimens in use). Study nurse clinicians, supervised by the doctors, are responsible for starting patients on the study drug. The doctors are consulted with respect to patients that require additional procedures.

Table 2 Summary of ART regimens currently in use at the Ubuntu Clinic and their routine monitoring

Regimen	Drugs	Monitoring Tests	Frequency
1a	d4T or AZT* /3TC/efavirenz	CD4	Baseline; 6-monthly
		VL	6-monthly
		ALT	Baseline; if symptomatic during follow-up
		FBC	Baseline
		Creatinine	Baseline
		HepB surface antigen (If positive, patient will receive Tenofovir)	Baseline
1b	d4t or AZT* /3TC/NVP	CD4	Baseline; 6-monthly
		VL	6-monthly
		ALT	Baseline; weeks 2,4 and 8, thereafter 6-monthly
		FBC	Baseline
		Creatinine	Baseline
		HepB surface antigen (If positive, patient will receive tenofovir)	Baseline
2	AZT/ ddI/ lopinavir/ritonavir	CD4	Baseline; 6-monthly
		VL	There is no monitoring for patients on Regimen 2 in the public health sector
		FBC	Baseline; then monthly for 3 months, then 6 monthly (with CD4 and viral load)
		Fasting cholesterol and triglyceride	Baseline; 6 months and then every 12 months
		Fasting glucose	Baseline and 12 months

Note: Baseline refers to testing done at initiation of ART. (Adapted from the South African National Antiretroviral Therapy Guidelines, 2004)

16 Follow-up phase: Intervention and post-intervention

Each follow-up visit includes screening for symptoms of active TB and drug adverse events using the symptom's screening checklist on the clinic case report form. The following information is obtained to assess adverse events other than TB: the presence of new nausea and/or vomiting, right upper quadrant pain, new rashes and new peripheral neuropathy. TB suspects during follow-up are investigated as described in the 'Ascertainment of active TB' section below; this is in line with guidelines provided by the South African National TB Program.

In the first few months of follow-up, there will be those patients who were initially assessed as being 'TB free' on the basis of a negative TB symptoms and signs screen but turn out to have positive baseline M.tb cultures. These will be regarded as definite cases of (prevalent) TB. On receipt of the TB results such patients will be contacted telephonically or by letter by study specific counsellors and recalled to the clinic. A clinical evaluation will be conducted and patients sent for chest radiography. Two sputum specimens will also be sent for TB microscopy and culture as well as for rifampicin and INH drug sensitivity testing before anti-TB therapy is commenced. Most of these patients will receive their anti-TB treatment at Ubuntu clinic.

Table 3 provides a summary of case definitions used to diagnose incident TB during follow-up. A toxicities-grading table is used to grade adverse effects (based on the Adult Clinical Trials Group table of toxicities).

Clinic patients who are on the study drug as well as ART are followed-up routinely by regular clinic nurses and doctors as well as study specific clinicians. The nurse practitioners refer problem cases where appropriate including TB suspects requiring investigations and those who report experiencing adverse events. Clinic doctors are also requested to alert the Study Coordinator of any TB suspects or any other adverse event being managed by them.

The lead investigator (MXR) and the Study Coordinator are responsible for ensuring that adverse events managed by regular clinic clinicians are followed up, that procedures are done per protocol, and that data collection tools are complete prior to data entering. At the end of each clinic visit, the Study Coordinator goes through all the clinic case records of identified study participants. These records are collected at Pharmacy or are left with the study team at the end of the clinic visit. The Study coordinator reviews these records for completeness, missed ALT determinations and for any missed adverse events including TB. Participants requiring additional procedures will then be recalled by study specific counsellors the following day. A diary (paper and electronic) of all participants returning for appointments is kept. Their folders are retrieved a day before their scheduled appointment and reviewed. Results are obtained and entered and the records scrutinized for any missed adverse events. Reviewing records pre and post-appointments is crucial for quality assurance.

16.1 Ascertainment of active TB during the follow-up phase

The study drug is interrupted during investigations for TB. Suspected pulmonary TB cases are sent for chest radiography and two specimens of sputum obtained for microscopy of acid-fast bacilli (AFB) by auramine staining and for mycobacterial culture. Sputum induction is performed on patients unable to expectorate spontaneously. Fine needle aspiration biopsy is performed in cases presenting with suspected TB adenitis or cold abscesses and the specimen sent for AFB smear and culture. Cases suspected with extra-pulmonary TB (EPTB) and requiring further investigations (e.g. lumbar punctures and ultrasonography) are referred to the G.F Jooste HIV Referral unit, our regional secondary level hospital. All suspected EPTB patients are requested to provide urine for AFB smear and culture. The study infectious disease physicians (GM & RJW) are consulted for difficult cases. Drug sensitivity profiling for isoniazid and rifampicin is requested in **all** culture positive incident TB cases (details described in Laboratory Assessment section below). The study coordinator is responsible for following up all results, tracking patients with positive results and notifying the primary attending doctor. Patients who were TB suspects and subsequently found not to have TB are restarted on the study drug provided they are not off the study drug for longer than 3months. TB treatment is commenced on all those who meet the case definition for TB (See Table 3). A record of number of interruptions and total time spent on and off the study drug per patient is kept. This information is recorded on the clinic case report form and is captured by both Pharmacy and study Data Capturers.

Table 3 summarising TB Case Definitions for incident TB

Definite TB	• Compatible clinical features plus one or both cultures is positive for M.tb
Probable TB	• Compatible clinical or radiographic features plus one or both smears positive for acid-fast bacilli (AFB) or histology positive for AFB or granulomatous disease on any specimen
Possible TB	• Compatible clinical features with radiographic features
Not TB	• If none of the conditions above are met

Note:
**Compatible clinical features include one or more of the symptoms or signs printed on the clinic case report form with or without any of the following: haemoptysis, shortness of breath, pleuritic chest pain, loss of appetite, extreme fatigue or lethargy, pallor or anaemia.*
***Compatible radiological features. Refers to films taken at the first assessment or during follow-up, up to 8 weeks since first presentation or until culture results are available*
****Response to antibiotics. Refers to a clinical and/or radiological response assessed two to eight weeks after the first presentation.*
***** The 2007 WHO diagnostic algorithm for extra-pulmonary TB will be followed.*

16.2 Summary of laboratory assessments and monitoring during follow-up

Routine Lab assessments:

ALT safety monitoring is determined at baseline and after 4, 8 and 12 weeks of study drug initiation and thereafter every 3 months (See Study Visit Schedule at the end of this section). Patients that are not bled for ALT on their clinic appointment are recalled to the clinic for phlebotomy. Otherwise, blood for ALT is taken at their next scheduled visit.

CD4+ lymphocyte counts & Viral load assessments:

These are done as per clinic protocol, six-monthly post ART initiation prior to 2010, then yearly following 2010. Virological failure is defined as a confirmed (within 3 months) viral load >1,000 copies/mL). The study drug is not interrupted during the management of virological failure.

Drug sensitivity testing for rifampicin and isoniazid resistance

The MGIT (Mycobacterial Growth Indicator System.) rather than the line probe assay has been adopted to test for resistance to both rifampicin and isoniazid in this study. The line probe assay misses about 15% of INH resistant strains as it only detects the common katG S315T and inhA C-15T mutations (pers comm, Robert Wilkinson).

17 Study Visit Schedule

	Screening	Enrolment	Intervention Period								Follow-up Period				
	-3 to -1	0	1-2	3	4-5	6	7-8	9	10-11	12	15	18	21	24	30**
1. INH/Placebo		X	X	X	X	X	X	X	X	X					
2. TB Screening	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
3. Adverse Event screening		X	X	X	X	X	X	X	X	X	X	X	X	X	
4. Education/Adherence counselling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
5. Clinician Visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
6. ALT*	X		X	X		X		X		X					
7. FBC	X														
8. CD4	X					X		X		X					
9. VL				X		X				X					

Note: *ALT monitoring at 0, 4, 8, 12 weeks then every 3 months during the intervention period. **Schedule in subsequent years of follow-up is the same as in the first year of follow-up. TB screening includes symptoms screening +/- chest radiography, CRP, sputum microscopy and culture. VL and CD4 determinations are done as per Ubuntu Clinic protocols.

18 Participant Retention

Measures to enhance participant retention include:

Managing and regularly updating contact details.

Locator information is obtained at baseline, two phone numbers and two physical addresses. The importance of keeping this information updated is reinforced at the waiting room mass education sessions and at each clinical visit. Participants that fail to turn up for scheduled appointments are telephoned by study specific counselors and a letter left at their place of residence or with a neighbour should they not be found. Home visits continue till the patient is traced or a decision taken to consider the patient as lost to follow-up.

Aligning study appointments with Ubuntu clinic schedules for ART patients

The visit schedule for patients commencing ART at the Ubuntu Clinic has been adopted. All patients new on ART attend the clinic on a monthly basis during their first year on therapy and those who are stable on ART (≥ 1 year on therapy) are given two-monthly clinical appointments. The decision to give two-monthly appointments is also dependent on a patient's individual circumstances (for example, those in employment and those migrating back to the rural areas on holiday). During the intervention period, the study team aims to conduct monthly study-specific assessments and monthly drug-refills. These visits largely coincide with ART drug collections. However, two-monthly appointments for study drug refills are scheduled for patients who have requested to collect ART on a two-monthly basis. Flexibility has been built into the trial's follow-up schedule to aid participant retention.

Counselling and education

Study participants, especially those new to ART, are encouraged to attend support groups organised specifically for patients newly on ART. These are conducted by regular adherence counsellors as well as those employed directly by the study to ensure continuity. Information on potential ART and study drug side effects will be

provided. Barriers to participation in the study as well as any myths regarding IPT or the study will be addressed at these forums. Information from these sessions will inform the design of a qualitative sub-study to be conducted.

19 Adverse events reporting and quantification

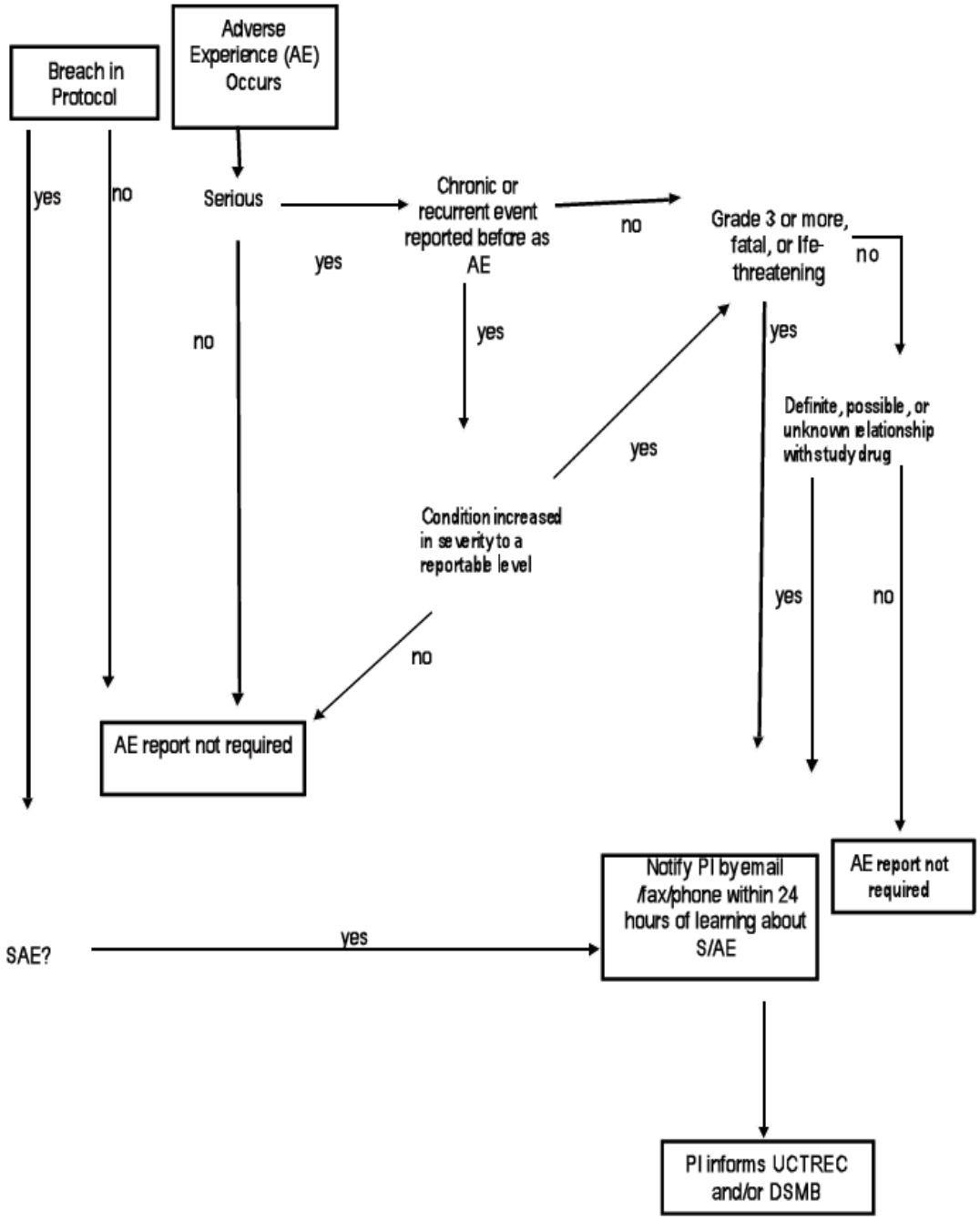
Definitions

Adverse Events (AE) includes all inter-current illnesses, unexpected events and breaches of protocol. However, the following are deemed as serious and therefore reportable to the Principal Investigator (PI): Development of active TB whilst on the study drug, AE grade 3 or 4 - Hepatotoxicity, Peripheral Neuropathy or Rash. AE of any grade which might lead to or results in permanent discontinuation of the study drug, AE of any grade which leads to permanent disability, AE of any grade which requires inpatient hospitalization, or any other grade which is considered serious and/or endangers the life of the patient, significant breach of study protocol, significant dosing error in study medication, death.

Reporting time-lines:

All expected and unexpected Grade3/4, fatal or life-threatening adverse events are reported to the PI within 24 hours of the study team having learnt of the event. This may be via email, fax or phone. AE not possibly related to the study medication are discussed with the PI. An AE form is completed for all expected and unexpected Grade 3/4 AEs. The PI is responsible for determining which reportable adverse events should be reported to the research ethics committee and/or DSMB. Please see the S/AE reporting flow chart below (next page).

ART- IPT study adverse events reporting flow chart



20 Monitoring, Diagnosing, Investigating and Managing Adverse Events

20.1 Important adverse events and their management

The national and provincial guidelines for managing patients on ART, already in use at the clinic are followed. All study-specific SOPs are based on national protocols. Toxicity is monitored primarily by symptoms – all patients are instructed on the symptoms of hepatotoxicity, peripheral neuropathy and rashes (the key overlapping adverse drug reactions of INH and ART). Nurse clinicians screen for TB and toxicity symptoms at each visit as described earlier. The specific evaluation and management of abdominal pain, elevated transaminases, peripheral neuropathy and rashes are explained briefly below.

Abdominal Pain

The differential diagnosis includes pancreatitis, hyperlactatemia, disseminated tuberculosis and hepatitis. Clinic protocols for evaluating these conditions are used. Investigations could include full liver function tests, amylase and serum lactate. If deemed necessary by the study clinicians, patients will be referred to GF Jooste Hospital for investigation and further management.

Hepatotoxicity

Abnormal transaminases are graded according to the ACTG-based toxicity table. The grading guides subsequent management as detailed in an SOP. Asymptomatic grade I/II elevations are monitored monthly without alteration of antiretrovirals or study drug. The study drug is permanently discontinued in all cases with Grade III/IV elevations of transaminases. The study drug is also permanently discontinued in all cases with symptoms or signs of clinical hepatitis regardless of the grade of transaminase elevation. These include: new nausea or vomiting, jaundice or right upper quadrant pain.

ART switches/interruptions for hepatotoxicity are according to existing protocols. Patients will be referred to GF Jooste for further evaluations if appropriate. The patients are followed-up until results revert to normal/baseline. Investigations for cases with suspected hepatotoxicity will include testing for Hepatitis B infection.

Peripheral Neuropathy

For grade I/II peripheral neuropathy (PN) reactions, all drugs are continued whilst also continuing careful clinical monitoring. The study drug is permanently discontinued for all worsening grade II PN. ART switches/interruptions for PN are according to existing protocols. Symptoms are controlled with analgesics, B6 or amitriptyline as appropriate. Details of management are described in an SOP.

Allergic Rashes

The grade assigned guides subsequent management. However, all cases are closely monitored in order to document deteriorations early. Both nevirapine and efavirenz may cause skin rashes but so can INH. Patients are examined for systemic symptoms including pyrexia. Liver function tests are ordered in all cases. A systemically unwell patient who presents with fever, rash involving mucosal surfaces, or abnormal liver function tests is permanently discontinued from the study drug. ART switches/interruptions for rashes are according to existing protocols.
The study drug is permanently discontinued for all deteriorating grade II reactions and for all grade III or worse reactions. A description of how to manage rashes is detailed in an SOP.

21 Non-adherence: definition, monitoring and managing.

Adherence is monitored through pharmacy refill records.

Study specific adherence counsellors organise adherence support groups for those patients on the study drug who are newly on ART. Patients established on ART are given the option to attend. Support groups are where patient concerns and problems are explored, study aims and processes revisited; danger signs of key adverse events and the importance of adhering to both the study drug and ART are discussed. Patients are expected to attend at least 3 support groups within the first 6 months ART. The counsellors report any urgent issues to the clinical team on a weekly basis.

Patients that are identified as poorly adherent (through pharmacy refill records) are referred for individualised counselling and reasons for poor adherence explored. If those reasons are deemed compatible with continuation of the study drug, the study drug is continued and an extra grace period of up to 3 months added to the intervention period. Patients that interrupt the study drug for more than 3 months will not receive further study drug, but will continue to be observed according to study protocols.

Patients who fail to turn up for appointments are telephoned on the day/the following day. Reasons for missing appointments are explored and the patients requested to attend the clinic as soon as it is convenient for them. If study staff are unable to reach them by telephone, a driver is sent out to deliver a letter at the provided residential addresses. The letter simply reminds the patients to attend the clinic without divulging any information on HIV or TB status. The process of telephoning and sending out a driver is repeated until an outcome is reached, for example, patient has moved or died.

22 Statistical Considerations

22.1 Initial sample size and power calculations

For the sample size estimations, assumptions are made with regard to the following:

The effect on ART on the overall rate of TB in patients who are newly starting ART and those established on ART.

The hazard rate of TB on ART reported for the Ubuntu HIV/TB clinic cohort is 0.12 (12 per 100 patient-years), a 59% reduction from its pre ART rate. (*pers comm.* Andrew Boulle). This reduction is slightly lower than a previous estimate from a cohort of mostly caucasian, men-who-have-sex-with-men in the city of Cape Town published by Badri and colleagues.(5) When this rate is stratified by CD4+ count for the same period on ART, the rate is 13,10,11 and 8.5 per 100 P-Y for CD4 categories 0-50, 50-100, 100-150 and >150 respectively (*pers comm.* Andrew Boulle). Stratification was done to explore the effect of CD4 on TB rate reduction.

Based on the above information, we assume the hazard rate of TB in the **control group** will be reduced to an average of **0.085 (8.5 per 100 patient-years)** during the study period (for both patients newly starting ART and those already established)**.** This might be an underestimate for new ART patients and perhaps a slight overestimate for patients established on ART.

The effect size in patients newly starting ART and those established on ART.

INH reduces the rate of active TB by 60% in TST positive HIV-infected people not yet on ART. This effect is reduced to less than 40% (RR=0.67) if PPD skin test status is not considered. We do not plan to use the skin test for randomisation based upon the evidence of TST and immunological surveys which suggests a high prevalence of latent TB in young adults in Cape Town townships like Khayelitsha.

We assume that INH plus ART combined will (conservatively) reduce the hazard rate of active TB in the intervention group by **35% to 0.055, (5.5 per 100 patient-years), a hazard ratio of 0.65.** We also acknowledge this might be an underestimate for those newly starting ART. The effect of co-prescription of ART and INH might be more pronounced in that group and they might potentially benefit more from this strategy of prevention as they would be receiving an additional preventative agent during their first year of ART whilst their immune system is still reconstituting.

Gains in CD4 only partially offsets the risk of TB

The median CD4 lymphocyte count at enrolment to the ART clinic in Khayelitsha is around 80 cells/mm3. CD4 count increases during follow-up potentially decrease the overall risk of TB in the cohort. However, recurrent TB in ART cohorts has been shown to occur even when substantial restoration of CD4 cell numbers has occurred.(22) (23) This is especially true in communities with a high prevalence of latent TB infection and continual re-infection such as Khayelitsha. Data from the Khayelitsha cohort has demonstrated high rates of TB across all CD4 baseline strata. We will assume that CD4 gain only partially offsets the risk of TB and thus does not affect sample size estimations.

Accrual patterns

Non-uniform accrual into the study is assumed where recruitment and follow-up of participants happens at the same time. No assumptions are made on the shape of the accrual distribution. The sample size is explored for various assumptions on accrual patterns: 1-year accrual with 2-year follow-up, 2-year accrual with 1-year follow-up, 2-year accrual with 2-year follow-up and 2-year accrual with a longer follow-up period of 3 years. We aim for a 1-year accrual and 2-year follow-up period.

Losses to follow-up due to withdrawals from the study and deaths.

It is assumed there will be exponential losses to follow-up throughout the study period, 10% in each study arm. Currently, about 5% of patients newly on ART are lost to follow-up in their first year of ART at the Ubuntu Clinic but that proportion rapidly increases with time on ART (25-30% by 3-4years follow-up on ART).

Subgroup analyses.

The sample size is inflated by 20% to ensure that study power would be maintained whilst conducting planned subgroup analyses. Further information on the pre-determined subgroups is given in the Statistical Analysis Plan section.

Statistical methods used to calculate sample size and power.

Further assumptions inherent in the methods chosen to estimate the sample size include: proportional hazards (hazard ratios not entirely dependent on time), Type II survival (the study is terminated after a fixed 3-year period when administrative censoring will occur, that is, regardless of whether all study participants have experienced a primary event or not), probability of Type 1 error of 0.05 and a probability of a Type II error of 0.20 (ie power of 80%). STATA 10 MP was used for estimating sample size and power. Methods are based on Lachin and Foulkes (Biometrics, 42, 507-519,1986).

Based upon our initial assumptions, a total **minimum sample size of 1204** was required for a 3-year study(1 year accrual and 2 years follow-up) to detect a 35% reduction in the hazard rates for tuberculosis in the intervention group (h_1= 0.052) compared to the control group (h_0=0.085) at a power of 80% and a Type I error of 0.05. See table 4 below. Our **maximum targeted sample size** when losses to follow-up and subgroup analyses are considered was **1445**. Table 5 detailing minimum and maximum sample sizes for a two-year accrual and two year follow-up period is provided below (next page).

Tables 4 and 5 showing minimum-maximum sample size required depending on the effect size, hazard rates in the control and the intervention group and assumptions on accrual and loss to follow up patterns. Type 1 error of 0.05 and 80% study Power.

Table 4 (Scenario initially aimed for)

One year accrual period and 2 year follow-up period

Rate in Control Group	Percentage Reduction	Rate in Intervention Group	Expected number of TB events. Sum of both arms	Minimum Sample Size. Sum of both arms.	Sample size plus LTFU	Sample Size plus LTFU and Subgroups
0.100	30%	0.070	252	1320	1488	1786
	35%	0.065	174	940	1058	1270
	40%	0.060	126	696	784	941
0.085	30%	0.060	242	1466	1652	1982
	35%	**0.055**	**172**	**1068**	**1204**	**1445**
	40%	0.051	118	756	852	1022
0.050	30%	0.035	252	2504	2828	3394
	35%	0.033	188	1902	2148	2578
	40%	0.030	126	1322	1494	1793

Table 5

Two year accrual period and 2 year follow-up period

Rate in Control Group	Percentage Reduction	Rate in Intervention Group	Expected number of TB events. Sum of both arms	Minimum Sample Size. Sum of both arms.	Sample Size plus LTFU	Sample Size plus LTFU and Subgroups
0.100	30%	0.070	252	1126	1300	1560
	35%	0.065	176	802	924	1109
	40%	0.060	126	594	684	821
0.085	30%	0.060	252	1300	1502	1802
	35%	**0.055**	**176**	**928**	**1072**	**1286**
	40%	0.051	126	686	792	950
0.050	30%	0.035	252	2112	2448	2938
	35%	0.033	174	1504	1742	2090
	40%	0.030	126	1116	1292	1550

22.2 Final sample size

On 1 June 2010 we obtained ethical approval to ammend our initial sample size estimates based on the following reasons:

a. Rates of loss to follow up are higher than we originally estimated (this results in a higher sample size)
b. Recruitment was slower than anticipated therefore there will be a longer follow up time for many participants. This will increase the number of tuberculosis events, which reduces the sample size.
c. The study will have limited power to conduct stratified subgroup analyses (making it inefficient to recruit large numbers). This reduces the sample size.

The nett effect of these three factors is that a minimum sample size of **1368** was required for a 4-year study (2 year accrual and 2 years follow-up) to detect a 35% reduction in the hazard rates for tuberculosis in the intervention group compared to the control group at a power of 80% and a Type I error of 0.05 (Lachin and Foulkes; Biometrics, 42, 507-519,1986).

23 Numbers needed to screen

Five to seven participants need to be enrolled onto the study drug daily at the clinic in order to complete recruitment within the time allowed by the study. Double the number of patients will have to be invited for screening in order to ensure the ~5 per day enrolment rate. This is based on assumptions that a certain proportion of patients will refuse to participate (~15%), others will agree but then never return to complete screening procedures (~10%) and others will fail to meet our pre-defined inclusion criteria at the baseline assessment visit. Those failing to meet the inclusion criteria will include patients new at the ART clinic who are already on anti-TB therapy, patients suspected of having TB (~15%) and other pre-specified non-TB related exclusions (~10%). Failure rates and reasons for failure will be reported at final analysis.

24 Statistical Analysis Plan

Results of this trial will be analysed and reported in accordance with the CONSORT and Pragmatic Trials in Healthcare groups guidelines for reporting pragmatic trials. (24) Intent-to-treat analysis (ITT) shall be conducted for both primary and secondary endpoints. A modified ITT will be considered where we shall omit from main analyses any randomized patients that withdraw from the study before receiving the study drug and those that are confirmed to have prevalent TB after they have been randomized. We are planning to conduct analyses during the study and once the study has been terminated. Analyses conducted during the trial will be DSMB-driven and also conducted to answer operational questions from blinded data. We shall explore how our baseline data, in particular, may be used to understand pertinent operational issues for ART programs planning to implement IPT. For example, exploring effective screening of HIV-infected persons on ART prior to IPT.

Main study analyses would be undertaken after the study has been terminated and unblinding procedures have been followed. DSMB-driven interim analyses and schedule are described in a later section.

24.1 Analyses after the study has been terminated.

A detailed description of the types of analyses and statistical tests for analysis of primary and secondary endpoints is provided below.

Primary Endpoint: Incident TB (Definite, Probable, Possible)

Cox proportional hazard rates for development of incident TB during the 3-year study period in the intervention and control arms will be compared as well as the influence (association; interaction) of pre-determined factors obtained at baseline and during the study follow-up. These include: Age, gender, ART duration, prior TB, viral load and CD4+ count (at baseline and most recent), ART regimen.

Participants who have not experienced the primary event of TB by the end of the study period will be ‘right censored’. The date of this administrative censoring will be

the day of study termination which will be determined by the PI. Patients lost to follow-up will be censored at the last clinic visit and we shall explore 1) whether there is a difference in proportions of those lost to follow-up between the study arms, and 2) how those lost to follow-up are different from the rest of the cohort (and between the arms) with respect to risk factors for TB at baseline. The date that TB develops will be considered to be half way between the date of diagnosis and the date last known to be event free. The date of TB diagnosis is the day the clinician obtains confirmation of TB diagnosis and decides to treat. The date of TB treatment will not be used for this as patients may get 'lost' in the system and may not start on the day the clinician has set.

Most patients attending the Ubuntu clinic for ART will present there as TB suspects and trigger investigation for TB. The study team will not miss those. However, there may be a few patients that will first present at a different clinic. In order not to miss these cases of tuberculosis we regularly ask all participants about treatment for tuberculosis and we will check all participant details in the provincial electronic tuberculosis register. We shall also be able to search the database of the National Health Laboratory Service for tuberculosis results as all specimens in the region are processed at a central reference laboratory.

Secondary Endpoints: Safety

Hazard rates for development of adverse events in the intervention arm compared to the control arm will be determined for the following variables: worsening grade II or grade III peripheral neuropathy, worsening grade II or grade III raised ALT, clinical hepatitis, unexpected serious adverse events. The median time spent within the milder grades during the intervention period per study arm would also be determined. Sensitivity analyses adjusting for baseline predictors (including ART type) emerging as strongly associated with a particular adverse event will be conducted. We shall also evaluate the risk of ART regimen change and factors that influence regimen change by study arms and also compare these with outcomes of the general clinic cohort.

Regression methods and survival type analyses will be used to determine the risk of poor adherence to ART and to the study drug as indicated by * pharmacy refills as well as the risk of *virological and/or *immunological failure in i) the control vs intervention arms, and ii) the combined study cohort outcomes compared to the outcomes of the general clinic cohort. Time to virological or immunological failure analysis will be explored.

*Please note definitions:
Pharmacy refill refers to scheduled drug-refill appointments. This variable will be evaluated as a continuous variable; *Virologic failure* is a 1-log (10 fold) increase in the lowest recorded level and *Immunologic failure* is defined as a 30% drop in CD4 count from peak-value, or a return to pre-ART baseline or lower. (SA National ART Guidelines, 2004)

Hazard rates for death will be determined as well as factors associated with mortality in i) the intervention arm compared to the control arm, and ii) the study cohort outcomes compared to expected outcomes in the general clinic cohort. The SA national death register will be used to search for patients that are lost to follow-up and not remaining in care at Ubuntu Clinic or any of the local HIV/TB clinics.

The study is not sufficiently powered to compare the risk of INH mono-resistance in microbiologically confirmed incident TB cases between the intervention arm and the

control arm (sample size results are omitted from this protocol). We shall, however, provide a descriptive report of proportions with any INH resistance as well as INH mono-resistance alone in the study arms and also include a description of subsequent TB treatment outcomes: TB treatment regimen, TB smear, culture and drug sensitivity testing results at two and five months post TB treatment (proportions failing to convert their smears or cultures are of interest) as well as the final outcome of whether the patient is cured or not and whether the multi drug regimen had to be intensified or prolonged to cover drug resistant strains.

24.2 Exploring operational issues for ART programs planning to implement IPT

Baseline screening data will be analysed to evaluate a TB screening tool used in the study, to describe case definitions for prevalent TB and to explore reasons for failing screening. These will be simple descriptive reports of the following:

- Proportions found to have TB (definite, probable or possible) or not depending on whether symptomatic or asymptomatic at baseline and according to our case definitions.
- Proportions of individual and combined signs and symptoms of TB at screening by ART status including sensitivity, specificity and predictive value tests, likelihood ratio tests and AUC analysis.
- Proportions that develop TB post initiation of the study drug in those initially diagnosed as 'not TB' will be described.

These will be related to pre-determined baseline characteristics that include age, gender, ART duration, prior TB, viral load and CD4+ count. Categorical variables will be compared by chi-square or Fisher exact tests and t and Wilcoxon rank sum test used for comparing continuous variables.

25 Ethical issues

Ethical approval has been obtained from the University of Cape Town Research Ethics Committee (REC Ref 013/2007). The ethical considerations are discussed below:

Why a pragmatic study design?

Dual epidemics of HIV and TB have promoted increased interest in conducting research in resource-poor countries. Such research should be responsive to local health needs and address urgent health concerns, increase capacity to provide quality service and should aim to achieve greater community involvement. The ART IPT trial is aimed as a model for conducting responsive HIV/TB research. The hypothesis, study outcomes and design are geared towards answering a key concern for ART programs: decreasing morbidity from TB. Trial processes will be integrated within clinic systems. Regular clinic staff will participate in all trial processes, including: protocol development, good clinical practice training, data management, education and counselling, recruitment and screening, participant follow-up and adverse events monitoring, drug-handling and defaulter tracking. Integrating trial processes with service provision at the clinic could be beneficial to both the research and the clinic and it specifically addresses questions around increasing capacity for

research and service provision. We believe a pragmatic design to clinical trials is best suited for advancing research responsiveness in developing countries.

Will the placebo group be unfairly 'denied' preventive therapy for TB?

We are proposing to administer INH to a group of individuals that is not currently given INH as preventive therapy for TB. All participants will be on ART as their primary therapy for reducing the risk of TB. Therefore, neither group will be unfairly compromised as they all receive ART. An unacceptably high incidence of TB and reinfection rates in the general community and amongst those already receiving ART justifies the attempt to seek further therapies that may further reduce the risk of developing TB in those on ART. The preventive role of ART in reducing the risk of TB in HIV infected persons is well documented; TB incidence is reduced by 60-80%. IPT reduces it by only 40% (in a population of unknown TST status). This is factored in the sample size calculation. IPT is currently not routinely implemented at stage I/II clinics in South Africa, therefore, the majority of patients commencing ART will not have been on IPT. The questions that clinicians are then faced with are: Will the addition of IPT further benefit these patients who are already receiving ART? And, Will they be at risk of combined toxicities if IPT is added? There is currently no evidence-based suggestion to adequately answer those questions.

Omitting the tuberculin skin test

We have opted to omit tuberculin skin testing as a tool to identify those who may benefit from IPT. This stance is supported by results from immunological surveys on latent TB infection that suggest there is a high prevalence of LTBI in township cohorts that is not detected by skin testing. TST or IGRA results will not be used to inform randomization. This means that there will potentially be participants with positive PPD skin tests who will be allocated placebo and ART, the current "standard of care". The SA guidelines support IPT for PPD+ HIV+ patients. However, as there is no evidence from RCTs showing additional benefit of IPT in patients on ART, this is currently not supported in the guidelines. Furthermore, the PPD+ rate will increase on ART.

It is not necessarily true that ART-augmented PPD+ will also need IPT. In PPD+ people the number needed to treat to avert a case of active TB would be about 20. The benefit may be considerably less in our study, therefore, the number to treat in this study could approach the number needed to harm (≥100). Hence we think there is enough equipoise on this matter for the trial to be conducted.

Note: NNT estimates (and assumptions) are taken from a review of treatment of latent tuberculosis infection in HIV infected persons conducted by Woldehanna and Volmink, Cochrane Database Syst Rev. 2000;(4):CD000171. These are summarized in the table below.

	Non-Active TB	Active TB	Absolute risk	Absolute risk difference % r1-r0, (95% CI)	NNT
PPD+					
Control	572	46	0.080	4.85	21
Experimental	675	18	0.027	(2.46 to 7.23)	
PPD-					
Control	1139	54	0.047	0.75	134
Experimental	1248	49	0.039	(-0.82 to 2.32)	

Isoniazid and adverse drug effects

Compared to alternatives, isoniazid appears to be relatively safe when combined with ART with respect to severe hepatotoxicities Hepatic transaminase levels will be monitored at regular time-points during the intervention period. Ubuntu clinic has the capacity to handle emergency cases as well as follow up patients who may have defaulted due to adverse events. SOP exists to guide the management of patients presenting with adverse effects.

Isoniazid mono-resistance

Government of the Western Cape reported that 8.6% and 5.7% of all HIV infected and uninfected patients (TB treatment naïve and re-treatment cases) starting TB treatment at Ubuntu clinic have INH mono resistance (Unpublished data-Personal communication, Gilles van Cutsem). INH mono-resistance may develop if patients who have active TB inadvertently receive INH mono-therapy. There is no prospective clinical data from similar communities to currently support or refute this. Reports remain anecdotal and some are based on modelling assumptions that may not hold true in real scenarios.(25) The sample size required to detect a difference in monoresistance rates between our two arms is large (results of sample size estimations are omitted from this protocol). A large systematic review of 13 IPT trials with ~35,000 participants failed to answer this question conclusively (RR 1.45, 95% CI 0.85-2.47).(26) The risk of isoniazid resistance remains a serious consideration in the study. Rigorous case detection methods for active TB are employed during study follow-up.

TB and drug adverse events early in anti-retroviral therapy

The first 3months of ART are associated with increased drug adverse events and increased risk of developing TB with or without immune reconstitution conditions. We have decided to commence patients newly starting ART on the study drug as early as it is possible in their therapy, based on a negative TB symptoms screen rather than waiting for culture results. No evidence exists with respect to whether the addition of IPT to ART may have an influence in reducing early incident TB cases and it is also not known whether this strategy will expose patients in the intervention arm to increased risks of drug adverse effects and INH mono resistance than those in the control arm. The decision to not wait for cultures increases the study's external validity in sub-Saharan Africa where culture is not widely available.

Non-adherence to study medication

Monitoring of adherence is done through drug-refills; participants failing to adhere to medication (ART and study drug) are managed according to clinic and study SOPs. It is also crucial to monitor whether co-prescription of the study drug adversely affects adherence to ART or ART outcomes.

The Data Safety and Monitoring Board

A Data, Safety and Monitoring Board is appointed to monitor increased adverse events and early effectiveness. The board will advise on early study termination if indicated. They will advise that the study be terminated when clear benefit or danger is observed in the intervention group vs the control group. Clear stopping rules and

early determination of outcomes have been developed. At interim analysis, the board will be in a position to advice investigators whether the study duration needs to be lengthened to increase study power or not, with consequent need to seek further funding. Further information on the DSMB is provided in a section below.

Informed consent form and information sheet

Written informed consent is obtained from all patients following a group counseling session. All elements of informed consent are discussed with potential participants in isiXhosa prior to any screening procedures and they are given a patient information leaflet to take home. These are again re-visited on the day of randomization. Consent forms were translated from English to isiXhosa and translated back to English again by a reputable professional translation company (Professional Language Bureau, Johannesburg, South Africa). Certificates are available upon request. Only the study specific Cousellors are tasked for educating, counselling and obtaining informed consent from potential participants and according to standard protocols.
(PIS and informed consent is attached)

Good Clinical Practice

Study information is explained to potential participants in a language comfortable to them and a study-specific consent form signed. The University of Cape Town's Research Ethics Committee and Medecins sans Frontieres' IRB have reviewed the protocol. The design of the study is robust and the sample size large enough to answer the primary endpoint. Adequate clinical evaluations and monitoring of safety laboratory parameters has been built into the design. Research and routine clinic staff is trained on Good Clinical Practice. This study will adhere to national and international guidelines of conducting clinical research and the Helsinki Declaration.

26 The independent data-monitoring committee and interim analyses

Statistical stopping rules and probabilities of detecting effectiveness or adverse events at interim analyses are available. A member of the DSMB has assisted in devising these rules. The DSMB monitoring schedule is briefly provided below:

1st monitoring point
Safety data will be sent to the DSMB when 25% of the total per-year (p-y) of followup has been completed. A difference of 50% or more for any specified adverse event between intervention and control group will be considered as important for the DSMB to take action and that may include stopping the trial early.

2nd monitoring point
Safety data as well as efficacy data will be sent to the DSMB when 50% of the total p-y has been accrued. A difference of 50% or more efficacy difference and for any specified adverse event between intervention and control group will be considered as important for the DSMB to take action and that may include stopping the trial early.

3rd monitoring point
Safety and efficacy data will be sent to the DSMB when 75% of the total p-y has been accrued. As in at other time points, an effect size of 50% or more will be considered as important for the board to take action.

27 Indemnities

Sponsor :University of Cape Town

Funders:Department of Health, South Africa, MSF Belgium, Wellcome Trust

Start date: 1 November 2007

Finishing date: 31 January 2012

28 References:

1. Woldehanna S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. Cochrane Database Syst Rev. 2004(1):CD000171.
2. Gordin FM, Matts JP, Miller C, Brown LS, Hafner R, John SL, et al. A controlled trial of isoniazid in persons with anergy and human immunodeficiency virus infection who are at high risk for tuberculosis. Terry Beirn Community Programs for Clinical Research on AIDS. N Engl J Med. 1997 Jul 31;337(5):315-20.
3. WHO. Strategic Framework to Decrease the Burden of TB/HIV. Geneva: World Health Organisation; 2004.
4. Azevedo V. City Health TB Coordinator. Cape Town; 2006.
5. Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. Lancet. 2002 Jun 15;359(9323):2059-64.
6. Boulle A. Tuberculosis in the context of ART. Case-finding in advanced HIV before and after the introduction of ART, and the impact of concomitant tuberculosis on ART outcomes. Second South African AIDS conference; 2005; Durban, South Africa; 2005.
7. Lawn SD, Badri M, Wood R. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. AIDS. 2005 Dec 2;19(18):2109-16.
8. Williams BG, Dye C. Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. Science. 2003 Sep 12;301(5639):1535-7.
9. Golub JE, Saraceni V, Cavalcante SC, Pacheco AG, Moulton LH, King BS, et al. The impact of antiretroviral therapy and isoniazid preventive therapy on tuberculosis incidence in HIV-infected patients in Rio de Janeiro, Brazil. AIDS. 2007 Jul 11;21(11):1441-8.
10. Quigley MA, Mwinga A, Hosp M, Lisse I, Fuchs D, Porter JDH, et al. Long-term effect of preventive therapy for tuberculosis in a cohort of HIV-infected Zambian adults. AIDS. 2001 Jan 26;15(2):215-22.
11. Mwinga A, Hosp M, Godfrey-Faussett P, Quigley M, Mwaba P, Mugala BN, et al. Twice weekly tuberculosis preventive therapy in HIV infection in Zambia. AIDS. 1998 Dec 24;12(18):2447-57.

12. Lugada ES, Watera C, Nakiyingi J, Elliott A, Brink A, Nanyunja M, et al. Operational assessment of isoniazid prophylaxis in a community AIDS service organisation in Uganda. Int J Tuberc Lung Dis. 2002 Apr;6(4):326-31.

13. ATS/CDC/IDSA. Treatment of tuberculosis. Am J Respir Crit Care Med. 2003(167):603-62.

14. Gordin F, Chaisson RE, Matts JP, Miller C, de Lourdes Garcia M, Hafner R, et al. Rifampin and pyrazinamide vs isoniazid for prevention of tuberculosis in HIV-infected persons: an international randomized trial. Terry Beirn Community Programs for Clinical Research on AIDS, the Adult AIDS Clinical Trials Group, the Pan American Health Organization, and the Centers for Disease Control and Prevention Study Group. JAMA. 2000 Mar 15;283(11):1445-50.

15. Gordin FM, Cohn DL, Matts JP, Chaisson RE, O'Brien RJ. Hepatotoxicity of rifampin and pyrazinamide in the treatment of latent tuberculosis infection in HIV-infected persons: is it different than in HIV-uninfected persons? Clin Infect Dis. 2004 Aug 15;39(4):561-5.

16. Breen RA, Lipman MC, Johnson MA. Increased incidence of peripheral neuropathy with co-administration of stavudine and isoniazid in HIV-infected individuals. AIDS. 2000 Mar 31;14(5):615.

17. Coetzee D, Hilderbrand K, Boulle A, Draper B, Abdullah F, Goemaere E. Effectiveness of the first district-wide programme for the prevention of mother-to-child transmission of HIV in South Africa. Bull World Health Organ. 2005 Jul;83(7):489-94.

18. Coetzee D, Hildebrand K, Boulle A, Maartens G, Louis F, Labatala V, et al. Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. AIDS. 2004 Apr 9;18(6):887-95.

19. Boulle A, Van Cutsem G, Cohen K, Hilderbrand K, Mathee S, Abrahams M, et al. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. JAMA. 2008 Aug 6;300(5):530-9.

20. Coetzee D, Boulle A, Hildebrand K, Asselman V, Van Cutsem G, Goemaere E. Promoting adherence to antiretroviral therapy: the experience from a primary care setting in Khayelitsha, South Africa. AIDS. 2004 Jun;18 Suppl 3:S27-31.

21. Boulle A, Bock P, Osler M, Cohen K, Channing L, Hilderbrand K, et al. Antiretroviral therapy and early mortality in South Africa. Bull World Health Organ. 2008 Sep;86(9):678-87.

22. Lawn SD, Bekker LG, Wood R. How effectively does HAART restore immune responses to Mycobacterium tuberculosis? Implications for tuberculosis control. AIDS. 2005 Jul 22;19(11):1113-24.

23. Girardi E, Antonucci G, Vanacore P, Palmieri F, Matteelli A, Iemoli E, et al. Tuberculosis in HIV-infected persons in the context of wide availability of highly active antiretroviral therapy. Eur Respir J. 2004 Jul;24(1):11-7.

24. Zwarenstein M, Treweek S, Gagnier JJ, Altman DG, Tunis S, Haynes B, et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. BMJ. 2008;337:a2390.

25. Cohen T, Becerra MC, Murray MB. Isoniazid resistance and the future of drug-resistant tuberculosis. Microb Drug Resist. 2004 Winter;10(4):280-5.

26. Balcells ME, Thomas SL, Godfrey-Faussett P, Grant AD. Isoniazid preventive therapy and risk for resistant tuberculosis. Emerg Infect Dis. 2006 May;12(5):744-51.

29 Appendix

The following are attached in a separate document:

Appendix A: ART IPT Study Project Plan

Appendix B: ART IPT Randomization Process

Appendix C: Screening algorithm

Appendix D: Survival curves showing time to the next TB diagnosis, pre ART and post ART

Appendix E: Exploration of power within subgroups