Isoniazid Preventive Therapy in HIV Infected Children on Antiretroviral Therapy Living in a High Tuberculosis Prevalence Area: a Randomized Controlled Trial

Thesis presented for the degree of

Masters of Philosophy – Paediatric Pulmonology

By

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Supervisor

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DECLARATION

I, Diane Gray, present this thesis in fulfilment of the requirements for the degree of Masters of Philosophy, Paediatric Pulmonology, in the Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town. The contents of this thesis are entirely the work of the candidate.

The candidate worked as one of the study doctors on the forerunner study 'Strategies for prevention of opportunistic infections in HIV-infected South African Children: Comparison of two Trimethoprim - Sulphamethoxazole prophylaxis regimens with and without concomitant Isoniazid - impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis study - a randomized controlled trial ' as well as the initial recruitment and follow up of the current project, for 18 months March 2005 until Dec 2007. The candidate subsequently became involved as a study co-investigator on the current project since March 2010 as part of a Discovery Foundation Academic Fellowship. She completed this concurrently with her sub-specialist training in Paediatric Pulmonology, through the Department of Paediatric Pulmonology, Red Cross War Memorial Children's Hospital, University of Cape Town. The study design was developed by the Principle Investigators Professor Heather Zar and Professor Mark Cotton with statistical and epidemiological support from Dr Carl Lombard. The candidate was involved in the implementation and running of the study including data safety and monitoring aspects of the study and data quality control. The candidate was involved in the study termination and finalising of data for analysis. The candidate was assisted in the analysis by Dr Carl Lombard (3rd author). The manuscript was written and edited by the candidate with input and comments from the co-authors including the supervisor and collated to form the final manuscript.

The work on which this thesis is based is original research and has not, in whole or in part, been submitted by myself or by any other person for another degree at this or any other university.

Signature and date: Signed by candidate

Signature removed

18 November 2012

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ABBREVIATIONS

ART - antiretroviral therapy

BCG - bacille Calmette-Guérin

CXR - chest radiograph

HIV - human immunodeficiency virus

INH – Isoniazid

HAART – highly active antiretroviral therapy

IPT - INH preventative therapy

MDR - multidrug resistant

PMTCT - prevention of mother to child transmission

PCR - polymerase chain reaction

RIF - rifampicin

SMX - sulfamethoxazole

TB - tuberculosis

TMP - trimethoprim

TST - tuberculin skin test

TU - tuberculin unit

WHO - World Health Organization

PART A: PROTOCOL

CURRICULUM VITAE OF SUPERVISER

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Zar, Heather Joy	Professor, Head and Director of Department of Paediatrics Child Health, University of Cape Town

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Witwatersrand, South Africa	MBBCh	1980-85	Medicine
Albert Einstein College of Medicine, LIJ Medical Center	Residency	1988-91	Pediatrics
Columbia University	Fellowship	1991-94	Pediatric Pulmonology
University of Cape Town	PhD	1998-00	Pediatric HIV-associated lung disease

A. Position and Honors.

Positions and Employment (last 5 yrs)

2010: Head of Department of Paediatrics and Child Health

2008: Professor and Chair School of Child and Adolescent Health, University of Cape Town

2007: Professor, University of Cape Town, School of Child and Adolescent Health

2007: Elected a Fellow of the University of Cape Town, South Africa

2006: Head of Department of Paediatric Pulmonology, School of Child and Adolescent Health, Red Cross Children's Hospital, University Cape Town

2002: Associate Professor, University of Cape Town, School of Child and Adolescent Health

Honors / awards (last 5 yrs)

2005: University of Cape Town Research award

2005: Best Senior Investigator, South African Thoracic Society

2005: Aspen Pharmacare Award from South African Thoracic Society

2005: Astra-Zeneca Research Award from South African Thoracic Society

2006: Finalist Department of Science, South Africa – Distinguished Woman in Science Award

2006: South African National Research Foundation B2 rated researcher as an internationally acclaimed researcher

2007: Fellow University of Cape Town

2009: Best publication award, South African Thoracic Society

2010: Special award from the International Pediatric Pulmonology Congress for "outstanding leadership and distinguished service to the children with the greatest need"

B. Selected peer-reviewed publications (in chronological order).

(Publications selected from over 100 peer-reviewed publications)

1. **Zar HJ**, Brown G, Donson H, Brathwaite N, Mann MD, Weinberg EG. Home made spacer devices for bronchodilator therapy in children with acute asthma – a randomized trial. Lancet 1999:354;979-82
2. **Zar HJ**, Weinberg EG, Binns HJ, Gallie F, Mann MD. Lung deposition of aerosol – a comparison of different spacers. Arch Dis Child 2000;82:495-498.
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53. **Zar HJ**, Pai M. Childhood Tuberculosis – a new era. Pediatr Resp Rev 2011;12(1):1-2
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Ongoing Research Support

1. National Institute of Health, USA, RO1, Zar (PI), 2008-2013
   Role: PI, Diagnosis of Tuberculosis in HIV-infected children – development of improved microbiological and immunological strategies RO1 HD058971-01
2. National Research Foundation, South Africa, Zar (PI), 01/01/2008-12/31/2011
   Incentive funding for rated researcher
3. National Research Foundation, South Africa, Zar (PI), 01/01/2007-12/31/10
   Role: PI, Molecular diagnosis of Pneumocystis pneumonia and emergence of resistance
4. Dept of Health, Research Program of Operational Plan for Comprehensive HIV Care, South Africa., Zar (PI), 01/06/2007–01/06/2011
   Role: PI, Strategies for prevention of opportunistic infections in HIV-infected South African children: comparison of 2 cotrimoxazole regimens with and without concomitant isoniazid

5. Medical Research Council, Zar (PI), 01/01/2006-12/31/09
   Role: PI, Long term study of 2 isoniazid (INH) prophylactic regimens with concomitant cotrimoxazole (CTX) in HIV-infected children – impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis
6. Wellcome Trust Strategic award WT084323MA, Wilkinson (PI), 2008-2013
   Role: co-investigator, A centre for clinical infectious diseases research at the University of Cape Town
7. Global Alliance for Vaccines and Immunization (GAVI), 01/10/2009-1/10/12
   Role: site PI, Case-control study on effectiveness of pneumococcal conjugate vaccine against pneumonia in HIV-infected and HIV non-infected children in South Africa.
8. National Institute of Health, USA, 01/9/09-1/9/2014
   Role: co-investigator, Tuberculosis Clinical Diagnostics Research Consortium. RFP-NIAID-NIH-AI2008026
9. European & Development Countries Clinical Trial Partnership (EDCTP), 2010-2013
   Role: Paediatric PI, Evaluation of multiple novel and emerging technologies for TB diagnosis, in smear-negative and HIV-infected persons, in high burden countries (the TB-NEAT study)".
10. National Institute of Health, USA, 01/9/11-2013
    Role: site PI, Optimal dosing of first line antituberculosis and antiretroviral drugs in children. R01 HD069175-01
11. Bill and Melinda Gates Foundation,2010-2016
    Role: PI, he Drakenstein Child Lung Health Study.

PROTOCOL

ISONIAZID PREVENTIVE THERAPY IN HIV INFECTED CHILDREN ON ANTIRETROVIRAL THERAPY LIVING IN A HIGH TUBERCULOSIS PREVALENCE AREA: A RANDOMISED CONTROLLED TRIAL

Protocol version 2, January 2010

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Background:

Tuberculosis (TB) is an important cause of childhood morbidity, mortality and death. The incidence of childhood TB has increased in low and middle income countries.[1] This resurgence is partly attributed to the coexisting burden of human immunodeficiency virus (HIV) disease [2], which is most pronounced in Sub-Saharan Africa. At the end of 2009 an estimated 33.3 million adults and 2.5 million children under 15 years were living with HIV.[3] In many HIV endemic areas there is a co-existing high TB prevalence.[4]

Dual infection with TB has an important impact on HIV disease. TB accelerates the progression of HIV disease by increasing viral replication.[5] It is a common cause of acute pneumonia in African HIV-infected children [6, 7] and frequently results in chronic lung disease including bronchiectasis.[8] TB is a common cause of death in HIV-infected children.[9] Antituberculosis drugs, such as rifampicin (RIF), have deleterious drug interactions with anti-retroviral therapy. Rifampicin, an inducer of cytochrome P450 CYP3A, decreases the concentration of both the protease inhibitors and non-nucleoside reverse transcriptase inhibitors, leading to sub-therapeutic levels, with an increased risk of inadequate viral suppression and drug resistance. The large pill burden of two multiple drug regimens increases the risk of adverse events such as liver toxicity and increases the likelihood of poorer adherence.[10]

Conversely, HIV infection impacts on TB disease. HIV infected children have a higher risk of developing primary TB as compared to seronegative children.[11] The clinical diagnosis of TB is more difficult in HIV-infected children as other opportunistic infections or HIV disease itself may mimic TB.

Furthermore tuberculin skin testing is less sensitive due to immunosuppression and chest radiography less specific.[9, 12] The outcome of HIV infected versus HIV uninfected children with TB co-infection is poorer, with mortality increased by six fold in HIV-infected children.[12, 13] The cure rate of TB in HIV infected children is significantly lower than that of HIV uninfected children [11, 12] and there is a higher rate of recurrence.[14] HIV-infected children stable on highly active antiretroviral therapy (HAART) are less likely to develop TB and have a better outcome than those not on HAART. However, the initiation of highly active antiretroviral therapy (HAART) in the setting of TB co-infection can lead to a paradoxical worsening of TB as a consequence of the 'immune reconstitution syndrome'.[15, 16]

Hence, preventing TB infection and disease in HIV-infected children is potentially an important public health intervention. Isoniazid (INH) has been used successfully as preventive therapy in HIV uninfected children at risk of TB disease.[17] Preventive therapy has been reported to be effective for prevention of TB disease in HIV-infected adults with a positive tuberculin skin test [18], reducing the risk of disease by 36%. A double blind placebo controlled trial investigating the efficacy of INH prophylaxis in HIV-infected children before HAART was widely available reported a significant impact on mortality and TB incidence - the mortality rate (32, 12.2%) was significantly lower in those on INH compared with placebo [11(8%) vs. 21(16%), HR 0.46 (95% CI 0.22 - 0.95), p=0.015], indicating a reduction in mortality of more than 50%. [18] The incidence of TB (10.8%) was also significantly lower in those on INH (3.8%) versus placebo (9.9%), HR 0.28 [95% CI 0.1 to 0.78], p=0.005), representing a 70% reduction in the incidence of TB. [18].

Thus INH may be an effective public health intervention for HIV-infected children living in high TB prevalence areas. However HAART itself protects against TB disease in adults and children. The efficacy of isoniazid preventive therapy (IPT) in preventing TB in the setting of wider availability of HAART for children in South Africa is not known. Other areas requiring further study include the long term protective efficacy of INH, the optimal duration of prophylaxis, and long term safety. Potential concerns with using IPT are the need to exclude TB disease in children before initiating INH preventive therapy and the impact this may have on antituberculosis drug sensitivity.

Aim:
To assess the efficacy, tolerability and safety of isoniazid preventive therapy compared to placebo in HIV-infected children on highly active antiretroviral therapy living in a high TB prevalence area.
Objectives:
To compare the impact of two different INH preventive regimens (daily or thrice weekly) on

- Incidence of culture confirmed and probable TB
- Mortality
- INH resistance with culture confirmed TB
- Incidence of adverse reactions
- Adherence

Method:
A longitudinal prospective double-blind placebo controlled trial comparing INH versus placebo in HIV-infected children attending Red Cross Children's Hospital or Tygerberg Children's Hospital. Enrolments began July 2006.
Inclusion criteria will be

- Weight ≥ 2.5kg
- Informed consent obtained from parent or legal guardian
- Resident in Cape Metropole
- Access to transport

- On HAART for ≥ 2 months with ≤ Grade 2 toxicity for liver enzymes and full blood count
- Adherence to HAART ≥ 90% for all components

Exclusion criteria will be:

- Chronic diarrhoea
- Current use of INH prophylaxis
- Prior hypersensitivity to INH
- Severe anaemia (haemoglobin less than 7 gm/dl)
- Neutropenia (absolute neutrophil count less than 400 cells)
- Thrombocytopenia (platelet count less than 50 000/ul)
- Non reversible renal failure
- Exposure to household TB contact

Tuberculosis will be excluded prior to randomization if not already done within the previous 3 months. Children will be screened for TB by history taking, Mantoux skin test, chest radiograph and 3 gastric washings or induced sputum when there are symptoms or new radiological abnormalities suggestive of TB. Any child found to have TB will be treated as per national guidelines; once treatment has been completed then INH or placebo may be initiated.

Children will be randomised at study entry to receive either INH or visually identical placebo either thrice weekly or daily according to variable blocked randomization lists prepared by the trial statistician. Pharmacists will label the trial drugs using sequential numbers from these lists. The dose of INH is 10 mg/kg/dose with a variability of 8-12 mg/kg determined according to whether half or quarter tablets are required. Placebo has an identical appearance to INH tablets and will be administered in a double blind manner.

Investigations

At the baseline visit a detailed history, examination and clinical HIV staging will be done, thereafter an abbreviated history and examination will be done at each visit. A full blood count (FBC), liver function tests (ALT) and urea and electrolytes (U&E) will be performed prior to randomization.

The absolute number and percentage of CD4 cells and viral load will be measured at study entry and then 6 monthly. A complete blood count and ALT will be performed 6 monthly. PPD skin testing will be repeated 6 monthly if prior tests are negative. A chest X-ray will be performed yearly to assess progression of lung disease; the X-ray will be reported according to a standardised format by a single radiologist who is blinded to the prophylactic regimen to which the child has been randomised.

At each visit, symptoms of adverse reactions to INH prophylaxis will be recorded. Reason for any hospitalization will be ascertained by the study team and recorded. Investigations for TB will include skin testing, chest X ray and gastric lavage or induced sputum specimens. The diagnosis of confirmed or probable TB will be made by the treating doctor and will be subject to review by 2 reviewers using the clinical, radiological and laboratory data.

Patients will be seen monthly for the first 6 months, then 2 monthly thereafter.

Adherence

Patients will be provided with an adequate medication supply and will be requested to return empty CTX and INH containers. Adherence for INH / placebo and CTX will be assessed through a 7-day recall. An adherence questionnaire will be completed three monthly. Adherence to INH prophylaxis will also be assessed by checking urine samples for INH metabolites yearly.

Development of TB during the trial or exposure to a household TB contact

- If a child develops pulmonary TB while on the study, the randomization code will be broken. If the child has been on placebo then 3 drug therapy will be commenced; if on INH then 4 drugs

will be used. Treatment will be modified according to sensitivities, should *M. tuberculosis* be cultured. If extra-pulmonary TB develops, subjects on placebo will be treated according to national guidelines; however, if randomized to INH a fifth drug will be added.

- The INH prophylactic regimen will be discontinued while the child receives treatment for TB but will be resumed once TB treatment has been completed.
- Children found to have TB will be notified and referred to their closest local TB clinic from where contact tracing is also performed
- HAART will be modified according to Provincial Guidelines
- If a household contact develops TB, the patient will be tested for TB (Mantoux skin test, chest X-ray and induced sputum or 3 gastric washings). If this occurs within 6 weeks of enrolment, investigations will not be repeated unless clinically indicated (weight loss, fever or cough for more than 2 weeks). If the child is not found to have TB, then open-label INH will be given daily for 6 months after which the INH/placebo will be restarted. The randomization code will not be exposed and the patient will continue assigned blinded therapy at the end of prophylaxis.

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PART B: LITERATURE REVIEW

ISONIAZID PREVENTIVE THERAPY IN HIV INFECTED CHILDREN

BACKGROUND

Tuberculosis (TB) is an important cause of childhood morbidity and death, particularly in high TB prevalence areas. In 2010 there were an estimated 8 million incident cases of tuberculosis globally, 1.2 million amongst people living with HIV.[19] The high prevalence of TB is driven in part by the HIV epidemic. At the end of 2010 an estimated 34 million adults and children were living with HIV, 68% of which live in Sub Saharan Africa.[3] TB and HIV frequently co-exist. The African region accounts for 26% of the global TB burden and 82% of the TB cases among people living with HIV.[19] In South Africa 60% of adult TB cases are living with HIV.[19] Although accurate TB epidemiological data is lacking in children due to difficulties in diagnosis and inadequate national notification, childhood TB is a reflection of the TB transmission within a community. Hence the burden of childhood TB can be expected to be large in areas with high TB prevalence.

Dual infection with TB has an important impact on HV disease. TB is associated with accelerated disease progression [20] and increased HIV viral replication.[5] Exposure of alveolar macrophages and lymphocytes from HIV-infected individuals to *M tuberculosis* in vitro leads to up-regulation of HIV viral replication. [5] HIV viral replication was enhanced in the broncho-alveolar lavage samples from TB involved as compared to uninvolved lung segments in HIV infected patients. [21] TB is a common cause of acute pneumonia in African HIV-infected children [22, 23] and often results in chronic lung disease or bronchiectasis.[8, 24, 25] TB is an important cause of death in HIV infected children as found in necropsy studies that predate highly active antiretroviral therapy (HAART).[25, 26] Tuberculosis drugs, such as Rifampicin, interact with antiretroviral therapy. Rifampicin is an inducer of cytochrome P450 CYP3A and decreases the concentration of the protease inhibitors, most notably ritonavir and to a lesser extent the non-nucleoside reverse transcriptase inhibitors (NNRTI). This leads to sub-therapeutic levels of the antiretroviral drugs and an increased risk of inadequate viral suppression and drug resistance.[27, 28] Therefore rifampicin should preferably be used with an NNRTI based regimen rather than protease inhibitor-based regimen. If a protease inhibitor is used, a ritonavir boosted protease inhibitor is required. In addition the large pill burden of two multi drug regimens may increase the risk of adverse events such as nausea and liver toxicity and the likelihood of poor adherence.[10]

Conversely, HIV infection impacts on TB disease. HIV infected children have a higher risk of developing primary TB as compared to HIV uninfected children.[11] A recent South African study reported a relative risk of developing culture-confirmed TB of 24.2 (95% CI 17 to 34) in HIV-infected versus uninfected infants.[29] Diagnosing TB in HIV infected children is difficult as other opportunistic infections and HIV itself my mimic TB. Tuberculin skin testing is less sensitive due to immunosuppression and chest radiographs are less specific. [12, 24] The outcome of TB in HIV infected children is worse than that of HIV uninfected children, with a 6 fold increase in mortality in the former.[12, 30] HIV infected infants and children with culture confirmed TB and poor access to HAART had a mortality of 21-39%.[29, 31, 32] HIV infected children have a lower cure rate than HIV uninfected children.[11, 12] In an Ethiopian cohort of children with TB, the cure rate was 58% for HIV-positive and 89% for HIV-negative TB patients.[12] There is a high rate of TB recurrence in HIV

infected children. In a South African cohort of HIV-infected children with culture confirmed TB, 16% had recurrent disease.[14] This is more than double the expected rate of TB recurrence of 2-7% in HIV uninfected patients with drug-susceptible TB who complete current standard short course tuberculosis therapy.[33] HIV-infected children on HAART are less likely to develop TB and have a better outcome than those not on HAART.[34-36] However the initiation of HAART in the setting of severe immunosuppression and TB-co infection can lead to a paradoxical worsening of TB, the immune reconstitution inflammatory syndrome.[16, 37, 38] Moreover the risk of TB in HIV infected children on HAART is still higher than that of HIV uninfected children.[39]

Preventing TB in HIV infected children is therefore desirable. Strategies to prevent TB infection in HIV infected children have included vaccination with Bacille Camille-Guerin (BCG) vaccine, isoniazid preventive therapy (IPT) and HAART. However the efficacy of BCG in HIV infected infants is not established [40] and may be reduced as HIV infected children have an impaired BCG-specific T-cell response in early life.[41] In addition BCG is unsafe in HIV infected infants with a high incidence of disseminated BCG disease and mortality.[42, 43]Therefore current WHO recommendations are that BCG should not be given to HIV infected infants at birth.[44] Isoniazid (INH), a TB medication, has been shown to be effective in preventing TB disease in HIV uninfected children exposed to TB [45], evidence which informs the current South African TB guidelines of secondary TB prophylaxis in children. These guidelines recommend INH prophylaxis for all children under 5 years who have a smear positive household TB contact. In addition they advise INH prophylaxis for all HIV infected children irrespective of age with a known contact due to the increased risk of TB disease as a consequence of HIV related immunosuppression. TB preventive therapy has been reported to be effective in HIV infected adults with positive tuberculin skin tests (TST), decreasing TB incidence by 36%[46], This protective effect was consistent for all regimens: INH, INH alone, INH with rifampicin (RIF), RIF with pyrazinamide (PZA) and INH, RIF and PZA. However there was no impact on mortality.[46] IPT is currently recommended therapy for HIV infected adults with a positive or unknown TST who live in TB endemic areas if TB disease is excluded.[47] Moreover in a large South African study in HIV infected adults, IPT given to TST positive adults significantly decreased TB risk in adults on HAART. HAART decreased the TB incidence by 64% (aHR=0.36, 95%CI 0.25-0.51) and IPT and HAART decreased TB incidence by 89% (aHR=0.11; 95% CI 0.02-0.78).[48]

In contrast to adults, preventing TB in children is aimed at preventing primary infection rather than reactivation disease. However, evidence of IPT efficacy in children is inconclusive. A recent Cochrane review of TB preventive therapy in HIV infected children concluded that INH prophylaxis in HIV infected children has the potential to prevent TB in HIV infected children, but that evidence was lacking to guide duration of preventive therapy, use of IPT in children on HAART or the long-term benefits and adverse events.[49] Further clarifying the impact of IPT in HIV infected children on HAART is important due to the potential to reduce the burden of TB disease and resultant morbidity and mortality in HIV infected children.

AIM

To review current evidence of IPT as a TB preventive strategy in HIV infected children

METHOD

A direct search of Medline database from 1980 to June 2012 through Pubmed was undertaken. The search terms included: (human immunodeficiency virus OR HIV OR human immunodeficiency syndrome) AND (tuberculosis OR TB) AND (preventive therapy OR chemoprophylaxis OR prophylaxis). The search was limited to human studies and ages 0 to 18 years. In addition reference lists of selected studies were reviewed for relevant information. The full search strategy can be followed in table 1.

RESULTS

Isoniazid preventive therapy (IPT)

Only four publications were found: one Cochrane review[49], two randomised placebo controlled trials (RCT) [39, 50] and one cohort study an extension of the Zar et al prospective placebo controlled trial [51]of IPT in HIV infected children. The Cochrane review included one study by Zar et al. The details of this study together with the other more recently published RCT and the cohort analysis are summarized in Table 2.

Impact of IPT on TB incidence and mortality

The study outcomes of TB and mortality risk of the three studies are summarised in table 3. The study by Zar et al was a randomized placebo controlled trial assessing the efficacy of INH in preventing TB in HIV infected children living in the Western Cape, South Africa, a TB endemic area.[50] This study reported a significant impact on mortality and TB incidence - the mortality rate (12.2%) was significantly lower in those on INH (8%) compared with placebo (16%), (HR 0.46 [95% CI 0.22 to 0.95], p=0.015), indicating a reduction in mortality of more than 50%. The incidence of TB (10.8%) was also significantly lower in those on INH (3.8%) versus placebo (9.9%), (HR 0.28 [95% CI 0.1 to 0.78], p=0.005), representing a 70% reduction in the incidence of TB.[50] The majority (91%) of children in this study were not on HAART at randomization as the study took place before HAART was widely available. Prior TB treatment was not an exclusion criteria, 16% of the cohort had been treated for TB prior to study enrolment. Eighty eight percent had severe immunosuppression at enrolment. The protective effect of INH occurred irrespective of TST status.

In contrast a second randomized placebo controlled trial of pre-exposure INH prophylaxis for TB in HIV infected and uninfected infants with perinatal HIV exposure, showed no decrease in TB risk between HIV-infected children receiving IPT and those receiving placebo.[39] This multicentre trial was set in South Africa and Botswana, both areas with high HIV and TB prevalence. The participants in this study differed significantly from the Zar et al cohort making direct comparison between the studies difficult (Table 2). The participants of the Madhi study compared with the Zar cohort were younger (3.1 versus 24.7 months), had no previous TB exposure (0 versus 16%), were less immunosuppressed (7.7% versus 88% with CDC stage B or C disease) and had better nutritional status (-0.6 versus -1.6 median weight for age z score) at randomisation. In addition in the study by Madhi et al 31% (versus 7.7%) of infants were on HAART at randomisation, with 99% (versus 31%) receiving HAART by study closure. The incidence in this cohort of 69 (16%) cases of TB highlights the large burden of TB disease in HIV infected infants in TB endemic areas despite early access to HAART.[39]

The cohort analysis of children enrolled in a randomized placebo controlled study of IPT (extended analysis of the Zar et al study) showed IPT to have an additional protective effect against TB in children on HAART.[51] This study by Frigati et al investigating the impact of dual IPT and HAART on TB disease in HIV infected children followed a cohort of 289 HIV infected children for a median of 21.7 months (IQR 9 to 27.4), equivalent to 495.7 person years of follow up. A combination of IPT and HAART reduced TB risk by 90% (HR 0.1, 95% CI 0.04 to 0.32) in HIV infected children. IPT alone reduced the risk by 78% (HR 0.22, 95%CI 0.09 to 0.53) and HAART alone by 65% (HR 0.32, 95% CI 0.07 to 1.55).[51] This study reported that advanced clinical disease and immunodeficiency at

enrolment was associated with increased TB disease risk. This study design and execution is limited by the fact that the subgroups were very variable in size and follow-up periods. This may have impacted on the statistical power and conclusions. However this is currently the only published paediatric study to assess the additional protective effect of IPT in HIV infected children on HAART.

Safety of IPT

Few adverse events were found and there was no difference in event rates between the groups receiving INH and those receiving placebo in these studies. Of the culture confirmed cases in the Madhi et al study, 1 of 11 (9%) cases with culture confirmed TB was INH resistant.[39] There were no cases of drug resistance in the 5 cases of culture confirmed TB in the Zar et al study.[50] There were 3 cases of INH resistance in the 19 cases of culture confirmed TB in the Frigati et al study, one of which had a drug resistant TB contact.[51]

Adherence

Adherence to primary IPT in HIV infected children was consistently high, 92 to 95% in these studies.[39, 52]

DISCUSSION

The current data suggests that IPT may play an important role in preventing TB infection and disease in HIV infected children on HAART living in high TB prevalence areas. The two studies investigating the protective effect of IPT in HIV-infected South African children have informed the WHO's newest recommendations on TB prevention and control.[47] These recommend that HIV-infected children older than one year of age receive IPT for 6 months irrespective of TB exposure or TST. IPT may be continued for up to 3 years in areas of high TB prevalence. Children less than one year of age who have been started on HAART for more than 3 months and are continuously screened for TB exposure should not receive IPT unless they have a documented TB exposure.[47]

The different results from the two randomized controlled studies may be explained by the different populations studied. Zar et al studied older children with previous TB exposure, more severe immunosuppression, more advanced disease and lower HAART coverage.[53] The Madhi et al study had good screening for and follow up of household TB contacts with any infant with a contact receiving INH prophylaxis and exiting the study.[39] This is a potentially important difference to explain lack of INH efficacy and may not reflect the reality of community settings, particularly in areas of high TB prevalence.[53, 54] TB contact tracing and initiation of IPT is very poorly executed in many TB programmes.[55] In a South African study 70% of 614 children exposed to culture positive TB were not given INH prophylaxis despite it being recommended management.[55]Older HIV infected children with delayed access to HAART who live in high TB prevalence areas should receive IPT based on current evidence. The efficacy of IPT in HIV infected children needs to be confirmed in larger randomised controlled studies powered to detect a significant difference in TB incidence amongst HIV infected children on HAART living in high TB prevalence areas. These studies should also be designed to address long-term tolerability and length of protective effect.

HAART has been reported to reduce the risk of TB disease in HIV infected adults [48] and children. [35, 36] A retrospective review of South African HIV infected children showed a TB incidence of 53.3/100 person years in the 9 months prior to starting HAART, which reduced to 6.4/100 person years in 13.5 months of post HAART follow up.[35] A multisite retrospective analysis of HIV infected children attending antiretroviral clinics showed a 3 fold decrease in TB incidence in HIV infected children on HAART compared to HIV infected children not on HAART. [34] However, HIV infected children on HAART still have a higher incidence of TB than HIV uninfected children.[39] In the Madhi et al study, despite HAART there was a high incidence of TB in the HIV infected infants over the 18.5 months of follow up: 12.1 cases per 100 child years in the HIV-infected compared to 4.1 cases per 100 child years in the HIV uninfected cases.[39] The study by Frigati et al shows IPT to have an additional protective effect over HAART, reducing TB risk by 77% in HIV infected children on HAART compared to those on HAART only. This cohort lived in a setting of high TB prevalence, were older than the cohort from the Madhi et al study, had more advanced immunosuppression at baseline and had a higher rate of previous TB exposure.[39, 51]

IPT has been shown to be safe in HIV infected children. In the studies reviewed there were few adverse events. Hepatotoxicity is a potential adverse event of INH therapy. [56, 57] Elevated serum transaminase levels occur in approximately 10% of children on IPT.[58, 59] Severe hepatotoxicity is less common[60]; progression to irreversible liver failure occurs in an estimated 3.2/100 000

children on IPT.[61] Anti-retroviral drugs are associated with a number of adverse events, in particular hepatotoxicity.[62, 63] Although antiretroviral drugs most often cause an asymptomatic elevation of transaminase levels, there have also been reported cases of fatal acute hepatitis in children in all classes of antiretroviral drugs. [62]Therefore there is concern about the safety of INH either used alone or especially concomitantly with HAART. IPT in African HIV infected adults was well tolerated with an INH associated toxicity incidence of 1.1%, an incidence rate the same as reported in HIV uninfected adults.[64] In a retrospective cohort study of liver toxicity in HIV-infected children receiving IPT, severe liver injury occurred in 5% of children.[65] In a recent study of primary IPT in HIV infected infants on HAART followed over nearly 2 years, the incidence of significantly raised ALT was 0.4% and significantly raised AST was 4%.[39] A prospective study in older HIV infected children enrolled in a randomised placebo controlled trial of INH prophylaxis reported a low incidence of hepatotoxicity in HIV infected children taking INH, with or without concomitant HAART. In this study 297 children on IPT were followed for 559 person years. Five (1.7%) IPT related episodes of severe liver injury occurred, an incident rate of 0.78 per 100 person years. [66] Younger age at start of IPT and higher baseline CD4 count were related to increased risk of developing toxicity.[66]

Definitive diagnosis of TB disease in children is difficult. Uncomplicated paediatric pulmonary TB is a pauci-bacillary disease so sputum smear microscopy is not a useful test. Culture of *M tuberculosis* is the confirmatory test but has a yield of only 20-40% in paediatric disease.[67] TST and interferon-gamma release assays are unable to distinguish TB infection from disease.[68] Clinical and radiological features are non specific but are commonly used in scoring systems to define probable disease. Many of these diagnostic scoring systems have been recently reviewed and found to significantly differ in diagnostic sensitivity.[69] Because of this difficulty there has been much concern that widespread IPT may lead to an increase in INH resistance as a consequence of inadvertent treatment of TB disease with INH alone. Data from the reviewed studies show no increase in drug resistant TB in HIV infected infants and children receiving IPT versus those receiving placebo. Review of adult HIV IPT programmes have found no increase in INH resistance amongst HIV infected adults treated with IPT and have consistently reported decreased TB risk.[48, 70, 71] The prevalence of drug resistance in a placebo controlled study of IPT in HIV infected and uninfected infants was 22.2% (95%CI 8.5-45.8) and INH mono-resistance 5.6% (95%CI 0.1-27.6) among culture-confirmed cases.[72] There was no association between INH or placebo, or HIV infection status and drug resistance.[72] The high incidence of drug resistance in this cohort is in keeping with recent reports of increasing drug resistance in paediatric TB cultures.[73] These studies support the safety of using IPT in HIV infected children but also the need to maintain surveillance for drug resistant TB in high prevalence settings. It is important that any IPT program includes a validated algorithm to reasonably exclude active disease before initiating IPT.

In the setting of rising TB drug resistance the importance of medication adherence is critical. A concern in the consideration of programmatic implementation of IPT in children has been the previously reported poor implementation and adherence to IPT given to TB contacts. Both the implementation of [55] and adherence to secondary INH chemoprophylaxis in children has been reported to be very low.[74] Only 20% of children receiving IPT after exposure to a household contact completed more than five months of INH.[74] However, adherence to primary IPT in HIV infected children has been consistently reported to be over 90% in paediatric studies.[39, 52] Adherence in these studies has been calculated through pill counts and verbal report, which have

limitations, particularly as there is no objective proof that the patient took the prescribed medication as it may have been discarded or shared.[75] This would give a false result of good adherence. Previous studies have reported using urine dipsticks to test urine INH metabolites, suggesting them to be a sensitive and an easy screening tool for INH adherence.[76, 77] However the sensitivity and specificity calculations were based on verbal and questionnaire report of adherence, neither of which are reliable measures of adherence themselves.[75] Paediatric data on the pharmacokinetics of INH and urine concentrations in children are lacking and would be useful in developing easy and more reliable screening tests for adherence to INH.

The optimal length of IPT in children to prevent either primary TB or TB re-infection is not known. A 6 month course of INH prophylaxis has previously been described to have a prolonged protective effect, in excess of 9 years, in HIV uninfected Alaskan adults. However, in high prevalence settings re-infection after TB prophylaxis or treatment is common.[78, 79] In adult IPT studies longer courses of INH were associated with decreased TB re-infection, suggesting that there is significant ongoing transmission in the community in high prevalence settings.[70, 80]

As long as the TB exposure within a community remains high preventing TB will remain a challenge. Hence if IPT is to be an effective preventive strategy it must be accompanied by every effort to reduce the ongoing transmission within the communities. This includes improved case finding, rapid diagnosis, appropriate treatment and infection control.[4] Preventing paediatric HIV infection through comprehensive prevention of mother to child transmission programmes is of critical public health importance. In addition early initiation of HAART in HIV infected infants decreases mortality and TB risk. [39, 81] Strengthening systems to improve TB case finding, contact protection and supervision of appropriate treatment and prophylaxis will help reduce the ongoing transmission within communities.

CONCLUSION

There is limited data on IPT in HIV infected children. INH has been shown to significantly reduce TB incidence and death in immunosuppressed HIV infected children not on HAART living in areas with high TB exposure. HIV infected infants receiving early HAART and with no known exposure to TB were not protected by primary INH therapy. IPT protects against TB disease in HIV infected children with advanced disease who are taking HAART.[51] The conflicting results from the aforementioned studies suggest that HIV infected children's level of immunosuppression, length of exposure and access to early HAART, as well as appropriate community screening and disease surveillance play a role in the risk of TB in HIV infected individuals and likely impact TB preventive therapy efficacy.

Further research is needed in order to clarify efficacy of INH as a prevention strategy in older HIV infected children on HAART living in high TB prevalence areas with limited resources. Studies should address issues of long term safety and tolerability, surveillance of drug resistance and length of protective effect. Research assessing TB systems' implementation of current guidelines and strengthening of disease surveillance, prevention and treatment is urgently needed in order to best use chemoprophylaxis safely and effectively.

TABLES

Table 1: Pubmed/Medline Search Strategy

Search Strategy	
#1	(("hiv"[MeSH Terms] OR "hiv"[All Fields]) OR (("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) AND immune-deficiency[All Fields]) OR (("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) AND ("immunologic deficiency syndromes"[MeSH Terms] OR ("immunologic"[All Fields] AND "deficiency"[All Fields] AND "syndromes"[All Fields]) OR "immunologic deficiency syndromes"[All Fields] OR "immunodeficiency"[All Fields]))
#2	(("tuberculosis"[MeSH Terms] OR "tuberculosis"[All Fields]) OR TB[All Fields])
#3	(("prevention and control"[Subheading] OR ("prevention"[All Fields] AND "control"[All Fields]) OR "prevention and control"[All Fields] OR ("preventive"[All Fields] AND "therapy"[All Fields]) OR "preventive therapy"[All Fields]) OR ("chemoprevention"[MeSH Terms] OR "chemoprevention"[All Fields] OR "chemoprophylaxis"[All Fields]) OR ("prevention and control"[Subheading] OR ("prevention"[All Fields] AND "control"[All Fields]) OR "prevention and control"[All Fields] OR "prophylaxis"[All Fields]))
#4	#1 AND #2 AND #3

Table 2: Comparison of included studies

Study	**Zar 2007[50] N=263**	**Madhi 2011[39] N=547**	**Frigati 2011[51] N=289**
Study design	Randomised placebo controlled trial	Randomised placebo controlled trial	Prospective cohort study set within a randomised placebo controlled trial of INH prophylaxis. Extension of Zar 2007 study.
Setting	Two health care settings in South Africa	Three South African and one Botswana health care centre	Two health care settings in South Africa
Intervention	INH (10mg/kg) or placebo given daily or three times a week	INH (10-20mg/kg) or placebo given daily	Jan 2003- May2004: INH (10mg/kg) or placebo; with and without ART June 2004 – Dec 2007: All children INH (10mg/kg) with and without ART
Exclusions	Known TB exposure requiring INH	Any current TB contact	Known TB exposure requiring INH
Recruitment	44% hospitalised	Hospitalised: very rare	Not reported
TB exposure on trial	Open label INH and resume study	Open label INH and exit study	Jan 2003- May2004: Open label INH and resume study June 2004 – Dec 2007: All children on INH
Details of participants and follow up			
Follow up – median (IQR)duration months	5.7 (2-9.7)	18.5 (0.25 - 27)	21.7 (9.5 - 27.4)
Participants	HIV infected children ≥ 8 weeks; n=276	Infants of HIV infected mothers; n=548	HIV infected children ≥ 8 weeks; n=298
Median (IQR) age in months at randomisation	24.7 (9.4-51.6)	3.06 (3 – 4)	25.1 (12.4 to 49.3)
Weight for age z score (IQR)	-1.56 (-2.5, -0.43)	-0.58 (-4.29, 3.07)	-1.34 (-2.41,-0.43)
CDC category B or C %	88 %	7.7%	Not reported
WHO CD4 category 3/4	Not reported	Not reported	83.2%
Median CD4 % (IQR) at randomisation	20 (14-28)	28 (6 – 58)	Not reported
Previous TB %	41/263 (16)	0 (exclusion criteria)	21/298 (7)
HAART			
ART at baseline n (%)	23/263 (9)	171/547 (31.5)	39/298 (13)
ART received during follow-up n(%)	81/263 (31)	541/547 (98.9)	174/298 (58.4)

Table 3 Comparison of outcomes of included studies

Study	Zar[50]	Madhi[39]	Frigati[51]
Death n (%)	INH: 11/132 (8%) Placebo: 21/131 (16%) HR 0.46 (95%CI: 0.22-0.95) *majority not on ART	INH: 27/273 (9.9%) Placebo: 17/274 (6.2%) HR 1.61 (95%CI: 0.88 to 2.96) *majority on ART	Not reported
TB incidence n (%) and/or Hazard ratio (95%CI)	INH: 5/132 (4%) Placebo: 13/131 (10%) HR 0.28 (0.1 to 0.78) *majority not on ART	INH: 31/273 (11.4%) Placebo: 38/274 (13.9%) *majority on ART	INH vs. placebo: HR 0.22(0.09,0.53) ART vs. placebo: HR 0.32 (0.07 to 1.55) INH+ART vs. placebo: HR0.11 (0.04 to0.32) INH+ART vs. ART: HR 0.23 (0.05 to 1)
Overall incidence of TB	30.6 cases per 100 children	121 cases per 1000 child years (95%CI, 95 to 153)	78 cases per 1000 child years
Culture confirmed cases	5/18 (28%)	11/69 (32%)	19/39 (48.7%)
Adherence: self report and pill count %(95%CI)	94.7% (95% confidence interval CI: 93.5-95.9)[52]	74-92%	>90% [52]
Adverse events	No difference between INH (5;4%)and placebo group (8;6%)	No difference between INH and placebo group	No difference between INH and placebo group
INH resistance	No cases in this cohort	1/11 (9%)	3/19 (15.8%)

PART C: PUBLICATION READY MANUSCRIPT

Isoniazid preventive therapy in HIV infected children on antiretroviral therapy living in a high tuberculosis prevalence area: a randomized controlled trial

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Contribution of the candidate
The candidate supervised and assisted in clinical work, data acquisition and preparation. The candidate undertook the data analysis and interpretation of data with assistance from biostatistician, CJ Lombard who is third author on the paper. She drafted the article and incorporated comments from co authors.

ISONIAZID PREVENTIVE THERAPY IN HIV INFECTED CHILDREN ON ANTIRETROVIRAL THERAPY LIVING IN A HIGH TUBERCULOSIS PREVALENCE AREA: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background

Tuberculosis (TB) is a common cause of morbidity and mortality in HIV infected children. Isoniazid preventive therapy (IPT) has been shown to reduce TB incidence in HIV infected children not on highly active antiretroviral therapy (HAART). Data on IPT efficacy in HIV infected children receiving HAART is inconclusive.

Aim

To assess the efficacy, tolerability and safety of isoniazid (INH) compared to placebo in HIV-infected children on antiretroviral therapy (ART) living in a high TB prevalence area.

Method

A randomised placebo controlled double blind study of INH was undertaken in HIV infected children on ART attending three centres in Cape Town, South Africa. Children were randomised to receive INH or placebo either daily or thrice weekly. Participants were prospectively followed from May 2005 to November 2011. The primary outcome measure was tuberculosis disease or death.

Results

One hundred and sixty seven children were randomised to receive INH (n=85) or placebo (n=82) and followed for a median of 34 months (IQR 24-52). The median age was 35 months (15-65) and median CD4% 27 (IQR 21-34). Six (4%) children had previous TB treatment and 14 (8%) previously received INH prophylaxis. There was 1 death in a child on INH and none in the placebo group. Eleven (6.6%) cases of TB occurred during the study period; 4 (5%) in the INH and 7 (9%) in the placebo group, incident rate ratio (IRR) for TB was 0.5 (95%CI: 0.15 to 1.75, p=0.284). Amongst the TB cases 5 were culture confirmed; 2 in the INH group and 3 in the placebo group of which all were sensitive to INH. Very few severe adverse events (6; 2%) occurred. This study observed a total of 278.5 patient years during which time only one case of INH related hepatotoxicity occurred.. Adherence was good in both groups. Dosing frequency had no impact on TB incidence and adverse events.

Conclusion

IPT is safe and well tolerated in HIV infected children on concomitant ART. INH showed a trend to protection against TB in HIV infected children on ART. These results support the need for a larger study to assess efficacy in HIV infected children on ART living in high TB endemic areas.

Background

Tuberculosis (TB) and HIV are dual epidemics and a public health crisis in Southern Africa. An estimated 2.5 million children are living with HIV, of which 2.3 million live in Sub-Saharan Africa, with an estimated 230 000 child deaths from AIDS. [3] In 2010 there were an estimated 8 million incident cases of tuberculosis (TB) globally, 1.2 million amongst people living with HIV. Over 1 million TB related deaths occurred, a third of which occurred amongst people living with HIV.[4] The African region accounts for 26% of the global TB burden and 82% of the TB cases among people living with HIV.[4]

TB and HIV are a deleterious combination. TB is a common cause of acute and chronic respiratory disease and a leading cause of death amongst HIV infected children in TB endemic areas.[23, 26, 82] TB accelerates HIV progression through increasing viral replication.[5] Diagnosing TB, which is difficult in children due to the pauci-bacillary nature of the disease, is even more so in HIV infected children.[12, 24] Both TB and HIV treatment require extended courses of multiple drugs increasing the likelihood of drug interactions, adverse events and poor adherence. Immune reconstitution during TB treatment or on initiation of combination antiretroviral therapy (ART) may lead to a paradoxical worsening of symptoms further complicating the diagnosis and management of TB in HIV infected children. Hence preventing TB and the subsequent immune deterioration is of major public health importance.

ART decreases mortality and reduce the incidence of opportunistic infections, including TB, in HIV infected adults and children.[35, 36, 83, 84] ART reduces TB risk and improves outcomes in HIV infected adults [48] and children living in high TB prevalence areas.[34-36] Although ART decreases TB risk, TB incidence in HIV infected as compared to uninfected infants and children remains high.[39]

Isoniazid preventive therapy (IPT) effectively prevents TB infection and disease in children exposed to a TB smear positive household contact.[45] TB preventive therapy reduces TB risk in PPD positive HIV infected adults by 36%.[46] In children the data are less clear. A randomized placebo controlled trial of INH prophylaxis in HIV infected children in the pre-ART era reduced all cause mortality by over 50% and TB by 70% in HIV infected children on INH.[50] However HIV infected infants receiving ART and with no previous exposure to TB received no protection from INH in a placebo controlled trial.[39] IPT offers additional protection against TB disease in HIV infected adults on ART [48]and is currently recommended by the WHO in all adults living with HIV in TB endemic areas.[4] In a cohort analysis of HIV infected children living in a high TB prevalence setting IPT offered additional protection against TB in older HIV infected children on ART.[51]

The current data suggest IPT may have a significant public health impact in older HIV infected children in high TB prevalence settings, but confirmatory data are lacking. This study aimed to assess the efficacy, tolerability and safety of IPT compared to placebo in HIV-infected children on ART who live in a high TB prevalence area to provide preliminary data.

Methods

A longitudinal prospective double-blind placebo controlled trial comparing INH versus placebo in HIV-infected children on HAART attending two hospitals and one community clinic in Cape Town, South Africa. Children were enrolled between May 2005 and Oct 2009 and followed until Nov 2011.

Participants

Participants were HIV infected children >8 weeks of age on ART for greater than two months. Inclusion criteria were: weight >2.5 kg, informed consent from a parent or legal guardian, resident in the area with access to transport and adherence to ART of >90%. Children were excluded if they had chronic diarrhoea, were currently using INH prophylaxis, had a history of prior INH hypersensitivity; had severe anaemia (haemoglobin less than 7 gm/dL), neutropenia (absolute neutrophil count less than 400 cells/µL), thrombocytopenia (platelet count less than 50 000/µL) or non reversible renal failure. Exposure to a household TB contact was also an exclusion criterion. Children with prior history of TB treatment or prophylaxis were eligible for inclusion. TB was excluded prior to randomization with symptom and contact history, tuberculin skin test (PPD, 2 TU RT23, Staten Serum Institut, Copenhagen, Denmark), chest radiograph and two induced sputa. Any child found to have TB was treated as per South African National TB treatment guidelines.[85] Once treatment had been completed the participant was then eligible for enrolment. Children were followed up two weekly for the first month, monthly for the first 6 months and then 3 monthly.

Allocation and prophylaxis

Within each of the three sites children were randomised at study entry to receive either INH or placebo either thrice weekly or daily according to variable blocked randomization lists prepared by the trial statistician. These lists were sent to the study pharmacist in sealed opaque envelopes. Participants were allocated a sequential number by the study nurse at enrolment and then sequentially allocated to treatment group by the pharmacist according to the pre-prepared lists. The dose of INH was 10 mg/kg/dose with a variability of 8-12 mg/kg determined according to whether half or quarter tablets were required. Placebo had an identical appearance to INH tablets and was administered in a double blind manner.

Investigations and study end points

At the baseline visit a detailed history, examination and clinical HIV staging was done. At follow up visits thereafter a symptom and contact history was taken and a physical examination completed. A full blood count (FBC), liver function tests (ALT) and urea and electrolytes were performed at baseline and FBC and ALT six monthly. The absolute number and percentage of CD4 cells and viral load were measured at study entry and then six monthly. PPD skin testing was repeated six monthly if the prior test was negative. A chest radiograph was performed. Additional chest radiographs were taken if there was concern of current TB disease or otherwise clinically indicated. The chest radiographs were reported according to a standardized format by a single radiologist who was blinded to the prophylactic regimen to which the child was allocated.

In addition, at each visit symptoms of adverse reactions to INH prophylaxis, details of intercurrent clinic or hospital visits were recorded. Diagnosis of TB: Children were classified as having *definite tuberculosis* if they were culture positive for *M tuberculosis*. *Probable tuberculosis* was diagnosed when chest radiography suggested tuberculosis (lymphadenopathy, military pattern, pleural effusion, bronchial compression, or parenchymal infiltrate) and the child had at least one of: a positive tuberculin skin test result, a history of a close contact with tuberculosis, loss of weight or failure to gain weight within the previous three months, or a positive smear result for acid fast bacilli. The diagnosis of definite or probable TB was made by the treating doctor and independently reviewed by an experienced clinician using the clinical, radiological and laboratory data and blinded to study randomization.

Development of TB during the trial or exposure to a household TB contact: If a child developed pulmonary TB while on the study, the prophylaxis was stopped. The child was placed on TB treatment in accordance with local guidelines (INH, rifampicin (RIF) and pyrazinamide two month intensive phase followed by INH and RIF for 4 months as continuation phase) and modified if necessary when sensitivities were available. Children taking lopinavir/ritonavir based ART had boosted ritonavir doses for duration of concomitant RIF therapy.[27] The INH/placebo prophylactic regimen was resumed once TB treatment was completed. If a household contact developed TB, the participants were investigated for TB disease (TST, radiograph and induced sputum or 3 gastric washings). Children without TB were given 6 months INH prophylaxis after which the assigned INH/placebo was restarted with blinding maintained.

Adherence

Patients were provided with an adequate medication supply and were requested to return empty INH containers. Adherence for INH / placebo was assessed through pill count of returned tables.

Statistical Analysis

This study followed after a larger study of INH prophylaxis in HIV infected children, most of who were not on HAART as the study started before its widespread availability. [50] Based on an incidence rate for TB in children from that study of 0.14/year (0.23 per year in placebo versus 0.07 per year in INH groups) a sample size of more than 300 children in each arm would have been required to detect a difference in the TB-free survival curves (assuming a more conservative hazard ratio of .5) between the groups with 90% power and a 0.05 level of significance. As this very large sample size was not feasible due to the funding and resource constraints available for the study, we planned to enroll 150 children to provide preliminary information on the efficacy, safety, tolerability and adherence to INH prophylaxis in children on HAART so as to inform a potentially larger study. All analyses were by intention to treat. We used Kaplan-Meier analyses to assess time to outcome, made comparisons with one sided log rank test and used Poisson and binomial regression models to estimate relative risks (incidence rate ratios for Poisson) of study outcomes: TB disease, adverse events and intercurrent events by randomization groups (drugs and regimen frequency) adjusted for stratification by site.

Consent

Written informed consent was obtained from the parent or legal guardian. This was undertaken in the parents' language of preference. The study was approved by the Research and Ethics Committees of the Faculty of Health Sciences, University of Cape Town (ethics no. 299/2005) and Stellenbosch University (ethics no. 2002C/073).

Results

One hundred and sixty seven children were enrolled at 3 sites (27 at Paarl TC Newman Hospital, 121 at Red Cross Children's Hospital and 19 at Tygerberg Hospital); 82 (49%) children were randomised to receive placebo and 85 (51%) to receive INH. There was 1 death in the INH group and 51 children were lost to follow-up: 24 could not be contacted, 24 relocated, 3 withdrew consent (Figure 1). The median follow up was 34 months (IQR 1 to 79). Baseline demographics were similar but the placebo group was six months older than the INH group, longer and heavier at baseline (Table 1). Both groups had similar CD4%, disease staging, ALT levels and viral loads at baseline. Twenty-six (16%) children had positive TST at enrolment, more in the placebo than treatment group. Six (4%) children had received prior treatment for TB and 14 (8%) had received INH prophylaxis (Table 1).

TB incidence: Eleven children developed TB during the study period, 7 (8.5%) in the placebo group and 4 (4.7%) in children taking INH. Five of the cases were definite TB, 2/4 (50%) in the INH group

and 3/7 (43%) in the placebo group; and 6 were probable TB cases. (Table 2) The total months of follow up for TB incidence was 2879 in the placebo group and 3276 in the INH group. This translates into 2.92 cases of TB annually per 100 children in the placebo and 1.46 cases of TB annually per 100 children in the INH group. The incident rate ratio for TB in INH as compared to the placebo group was 0.51 (95%CI: 0.15 to 1.75, p=0.284. Children taking medication daily had more TB events 7 in 3295 person years (2.5 cases annually per 100 children) as compared to those taking medication thrice weekly 4 in 2860 person months (1.7 cases annually per 100 children) / with an IRR 1.54 (95% CI: 0.45 to 5.27; p=0.49). Most TB events (8; 73%) occurred during the first 18 months of the follow up, including all 7 events in the placebo group. During the subsequent follow up (months 19 to 79) there were 3 more events in children taking INH (Figure 2).

Adherence: Adherence was stable and good throughout the study (Table 3). The mean adherence was 97% (SD 6.8). The mean adherence in the placebo group was 97.8% (SD 6.4) compared to 96% (SD 7) in the INH group, p=0.06. The adherence in the group taking medication daily was slightly better than that in the thrice weekly group, 98% compared to 95.8% (P=0.04). There was no interaction between treatment and dosing schedule when accounting for factorial design (p=0.697,). Adherence had no association with TB outcome (IRR 0.97, 95%CI 0.87-1.07, p=0.578).

Adverse events: There were very few severe adverse events (Table 3). Four of 6 events were temporary transaminitis, three of which were unrelated to study medication. One case of transaminitis may have been related to study medication, when medication was stopped, transaminitis settled and the medication was safely reintroduced. There was one case of trimethoprim-sulphamethoxazole hypersensitivity rash and one case of premature telarche, neither of which were related to the study medication. Taking into account follow up time, intercurrent open label prophylaxis of placebo assigned participants and intercurrent TB treatment, this study observed a total of 278.5 patient years of daily or thrice weekly INH in HIV infected children on HAART. During this time only one case of INH related transaminitis occurred.

Hospital admissions: There were 53 intercurrent hospitalizations in 29 children during study follow up, 13 children in the placebo group and 16 children on isoniazid, p=0.68 (Table 3). The relative risk of hospitalization for isoniazid versus placebo was 1.34 (95%CI 0.74-2.4, p=0.3) and for dosing regimen, daily versus thrice weekly 1.22 (95%CI 0.69-2.21, p=0.48).

Discussion

This study addresses the impact of INH on TB disease in an important group of HIV infected children, namely older children on ART living in areas of high TB prevalence. In this study the TB incidence in the placebo group, compared to the INH group (2.8 versus 1.5 cases annually per 100 children) suggests an INH protective effect. Larger studies are needed to assess INH efficacy in this patient population. No difference in TB incidence between children receiving prophylaxis daily compared to 3 times a week occurred, similar to the findings of a prior study in which the prophylaxis dosing regimens of daily or 3 times weekly INH had similar efficacy.[50] The TB incidence rates in the placebo and INH groups are much lower than previously reported by Zar et al in a prior study of HIV infected children in the same region (23.4 and 7.2 cases annually per 100 children in HIV infected children receiving placebo and INH respectively) however most children were not on ART.[50] This result is consistent with the protective effect of ART against TB. In addition the children in this study differed from the previous studies of IPT in HIV infected children. Compared to the Zar et al study

the children were older, all on ART and better nourished.[50] All three studies were set in high TB prevalence areas but, in contrast to the Madhi et al study, where close surveillance of and use of INH in any infant with a TB contact was undertaken, children in the current study relied in part on TB contact tracing and management from local TB programmes.[39] TB contact tracing and management has been shown to be poor in many TB programmes.[55, 74] As with the Zar et al study, the current study more closely represents the real life conditions and exposures of HIV infected children in high TB prevalence areas. Of the 4 cases of TB in the INH group, only one case occurred within the first 18 months of follow-up. This may suggest a waning in protective effect of INH after 18 months.

The main limitation of this study is the small sample size providing inadequate power to detect a significant difference in TB incidence between the INH and placebo groups. Diagnosing TB in children is difficult due to the pauci-bacillary nature of the disease, with culture of *M tuberculosis* being the definitive test. Only 5 cases of culture confirmed TB occurred. The use of diagnostic algorithms is limited [69] and it is conceivable that some of the children diagnosed with probable TB may not have had TB. However the diagnosis of TB was reviewed by an independent paediatric TB expert who was blinded to randomisation and all were treated for TB with clinical improvement.

All cases of culture-confirmed TB were sensitive to INH, providing evidence of its safety when used long term and is consistent with prior studies of IPT in HIV infected children have reported similar results.[39, 50, 51] In a study of IPT in HIV infected and uninfected infants there were 5 cases of INH resistance amongst 19 cases of culture confirmed TB, 2 of which occurred in the INH group and 3 in the placebo group.[39] Large adult studies of IPT have shown no increased risk of INH resistant TB in HIV infected adults taking IPT.[48, 70, 71] Surveillance must however be maintained and a validated algorithm to exclude active TB disease followed before initiating IPT.

Adherence in this cohort was excellent, consistent with previous reports of primary IPT in HIV infected children in IPT studies.[52] These adherence rates are much higher than those described with secondary INH prophylaxis in which only 36% of children completed the prescribed 6 months of INH.[74] IPT adherence may be lower in a programmatic setting. However these HIV infected children taking IPT already had excellent adherence to HAART regimens, which may have facilitated good adherence to IPT. In addition caregivers may have seen the IPT as part of a regimen for treating an illness rather than perceiving the more difficult concept of a preventive strategy.

A strength of this study is the long time of follow-up, during which there was only 1 episode of INH related hepatotoxicity. This rate is even lower than the recently reported incident rate of 0.78 per 100 person years in another INH prophylaxis placebo controlled trial in older HIV infected children.[66] This is consistent with previous reports of IPT safety in HIV infected infants and children on HAART.[39, 65]

In conclusion this study, the first randomised controlled trial to assess INH preventive therapy in older HIV infected children on HAART, showed a trend to protection against TB. INH is safe and well tolerated in HIV infected children on concomitant HAART. These results support the need for a larger study to assess efficacy in older HIV infected children on HAART living in high TB prevalence areas.

Figure 1: Enrolment, randomization and follow-up of study cohort

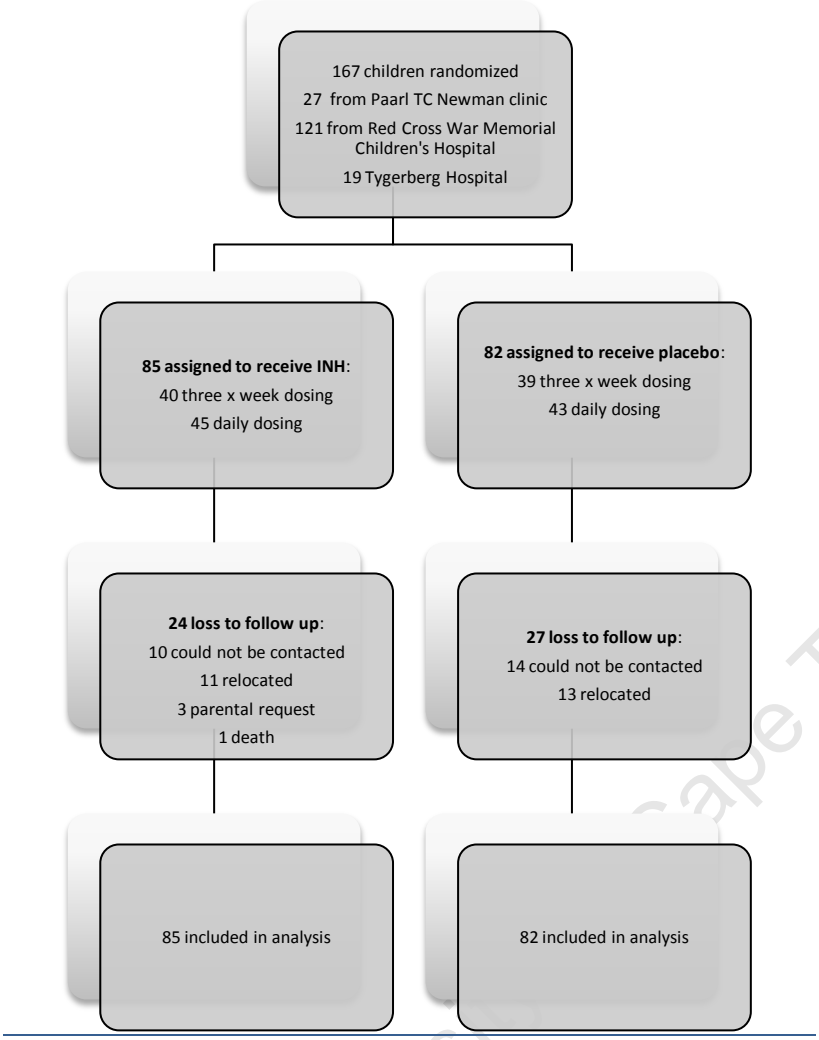

Figure 2 Time to TB diagnosis in the INH and placebo groups

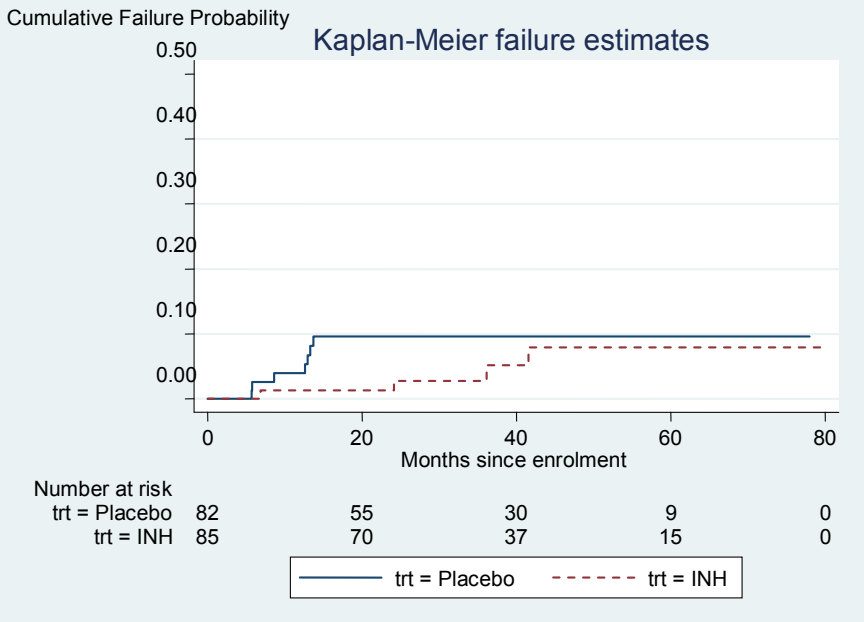

Table 1 Baseline demographic data for children

	Placebo	INH
N (%)	82 (49)	85(51)
Dosage daily n (%)	38 (48)	40 (47)
Male n (%)	42 (51)	41 (48)
Age in months median (IQR)	38 (15 to76)	32 (17 to63)
CD4 count median (IQR)	1159 (866 to1457)	1147 (783 to1749)
CD4% median (IQR)	27 (22 to34)	27 (19 to33)
Weight for age z-scores median (IQR)	-0.95(-1.72 to-0.19)	-1.11(-2.3 to -0.25)
Height for age z-score median (IQR)	-1.22(-2.3 to-0.6)	-1.41 (-2.67 to-0.37)
Weight for height z-score median (IQR)	0.12(-0.44 to-1.08)	0.23(-0.57 to 0.82)
ALT[1] mean (sd)	22 (9)	24 (16)
HIV viral load LDL[2] n (%)	58 (69.5)	57 (67.0)
HIV viral load log median (IQR)	0 (0 to670)	0 (0 to530)
WHO[3] classification		
Stage 2 n (%)	6 (7)	6 (7)
Stage 3 n (%)	33 (40)	37 (44)
Stage 4 n (%)	43 (52)	42 (49)
Months on ART median (IQR)	34.0 (19.8 to 49.8)	31.5 (21.8 to 50.5)
TST[4] positive n (%)	14 (17)	12 (14)
Previous TB treatment n (%)	4 (5)	2 (2)
Previous TB prophylaxis n (%)	8 (10)	6 (7)

[1]alanine transferase, [2]lower than detectable level, [3]World Health Organisation, [4]tuberculin skin test

Table 2 Incidence of TB in children

	Placebo	INH
	n=82	n=85
Mean months of follow up (sd)	35 (19.5)	38.5 (21.4)
Total months of follow up	2879	3276
TB incidence n (%)	7 (8.5)	4 (4.7)
Confirmed TB n (%)	3 (43)	2 (50)
Probable TB n (%)	4 (57)	2 (50)
Frequency of dose		
Thrice weekly	2/39 (5)	2/40 (5)
Daily n (%)	5/43 (11)	2/45 (4)

Table 3 Adherence, intercurrent hospital admissions and adverse events in children by dosing schedules

	INH n=85	**Placebo n=82**		**Daily (n=88)**	**Thrice weekly (n=79)**	
Adherence % mean(SD)	96 (7)	97.8 (6.4)	P=0.06	98 (7.3)	95.8 (6)	P=0.04
			Relative risk (95%CI)			Relative risk (95%CI)
Hospital admissions n (%)	16 (55)	13 (45)	1.22 (0.64-2.35)	13 (45)	16 (55)	0.69 (0.36-1.32)
Adverse events	4 (67)	2 (33)	Fisher exact P=1.0	3 (50)	3 (50)	Fisher exact P=1.0

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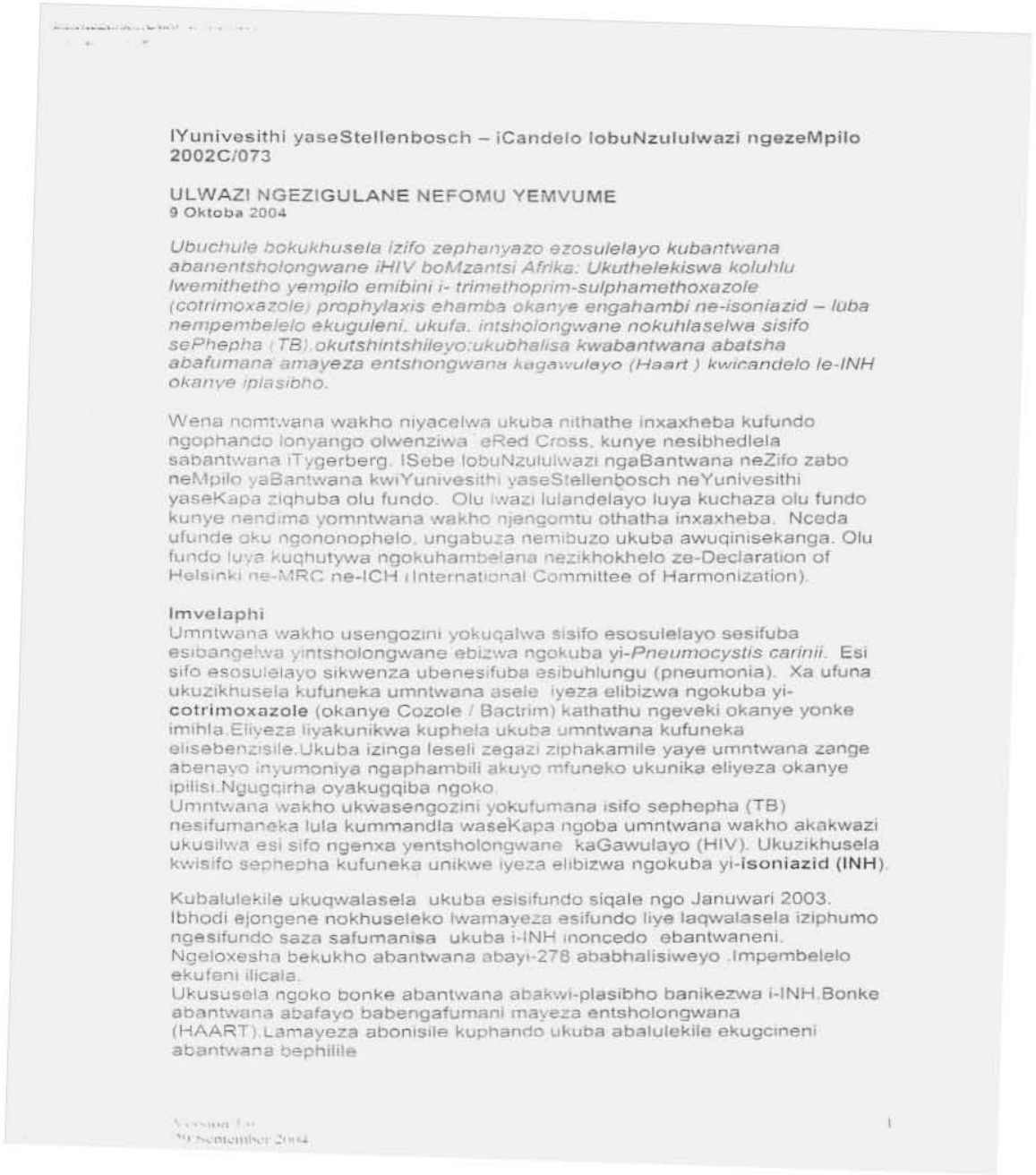
PART D APPENDICES

Appendix 1 Consent and Information forms



IYunivesithi yaseStellenbosch – iCandelo lobuNzululwazi ngezeMpilo
2002C/073

ULWAZI NGEZIGULANE NEFOMU YEMVUME
9 Oktoba 2004

Ubuchule bokukhusela izifo zephanyazo ezosulelayo kubantwana abanentsholongwane iHIV boMzantsi Afrika: Ukuthelekiswa koluhlu lwemithetho yempilo emibini i- trimethoprim-sulphamethoxazole (cotrimoxazole) prophylaxis ehamba okanye engahambi ne-isoniazid – luba nempembelelo ekuguleni, ukufa, intsholongwane nokuhlaselwa sisifo sePhepha (TB),okutshintshileyo;ukubhalisa kwabantwana abatsha abafumana amayeza entshongwana kagawulayo (Haart) kwicandelo le-INH okanye iplasibho.

Wena nomtwana wakho niyacelwa ukuba nithathe inxaxheba kufundo ngophando lonyango olwenziwa eRed Cross, kunye nesibhedlela sabantwana iTygerberg. ISebe lobuNzululwazi ngaBantwana neZifo zabo neMpilo yaBantwana kwiYunivesithi yaseStellenbosch neYunivesithi yaseKapa ziqhuba olu fundo. Olu lwazi lulandelayo luya kuchaza olu fundo kunye nendima yomntwana wakho njengomtu othatha inxaxheba. Nceda ufunde oku ngononophelo, ungabuza nemibuzo ukuba awuqinisekanga. Olu fundo luya kuqhutywa ngokuhambelana nezikhokhelo ze-Declaration of Helsinki ne-MRC ne-ICH (International Committee of Harmonization).

Imvelaphi
Umntwana wakho usengozini yokuqalwa sisifo esosulelayo sesifuba esibangelwa yintsholongwane ebizwa ngokuba yi-*Pneumocystis carinii*. Esi sifo esosulelayo sikwenza ubenesifuba esibuhlungu (pneumonia). Xa ufuna ukuzikhusela kufuneka umntwana asele iyeza elibizwa ngokuba yi-**cotrimoxazole** (okanye Cozole / Bactrim) kathathu ngeveki okanye yonke imihla.Eliyeza liyakunikwa kuphela ukuba umntwana kufuneka elisebenzisile.Ukuba izinga leseli zegazi ziphakamile yaye umntwana zange abenayo inyumoniya ngaphambili akuyo mfuneko ukunika eliyeza okanye ipilisi.Ngugqirha oyakugqiba ngoko.
Umntwana wakho ukwasengozini yokufumana isifo sephepha (TB) nesifumaneka lula kummandla waseKapa ngoba umntwana wakho akakwazi ukusilwa esi sifo ngenxa yentsholongwane kaGawulayo (HIV). Ukuzikhusela kwisifo sephepha kufuneka unikwe iyeza elibizwa ngokuba yi-**isoniazid (INH)**.

Kubalulekile ukuqwalasela ukuba esisifundo siqale ngo Januwari 2003. Ibhodi ejongene nokhuseleko lwamayeza esifundo liye laqwalasela iziphumo ngesifundo saza safumanisa ukuba i-INH inoncedo ebantwaneni. Ngeloxesha bekukho abantwana abayi-278 ababhalisiweyo .Impembelelo ekufeni ilicala.
Ukususela ngoko bonke abantwana abakwi-plasibho banikezwa i-INH.Bonke abantwana abafayo babengafumani mayeza entsholongwana (HAART).Lamayeza abonisile kuphando ukuba abalulekile ekugcineni abantwana bephilile

Version 1.0
30 September 2004

1

2002C/073

PATIENT INFORMATION AND CONSENT FORM
May 23rd, 2003

Strategies for prevention of opportunistic infections in HIV-infected South African children: Comparison of 2 trimethoprim-sulphamethoxazole (cotrimoxazole) prophylaxis regimens with and without concomitant isoniazid – impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis.

You and your child are requested to participate in a medical research study that is being done at Red Cross and Tygerberg Children's hospitals. The Department of Paediatrics and Child Health of the Universities of Cape Town and Stellenbosch are conducting this study. The following information will describe the study and your child's role as a participant. Please read this carefully and feel free to ask any questions. The study will be conducted according to the Declaration of Helsinki and to MRC and ICH (international committee of harmonization) guidelines.

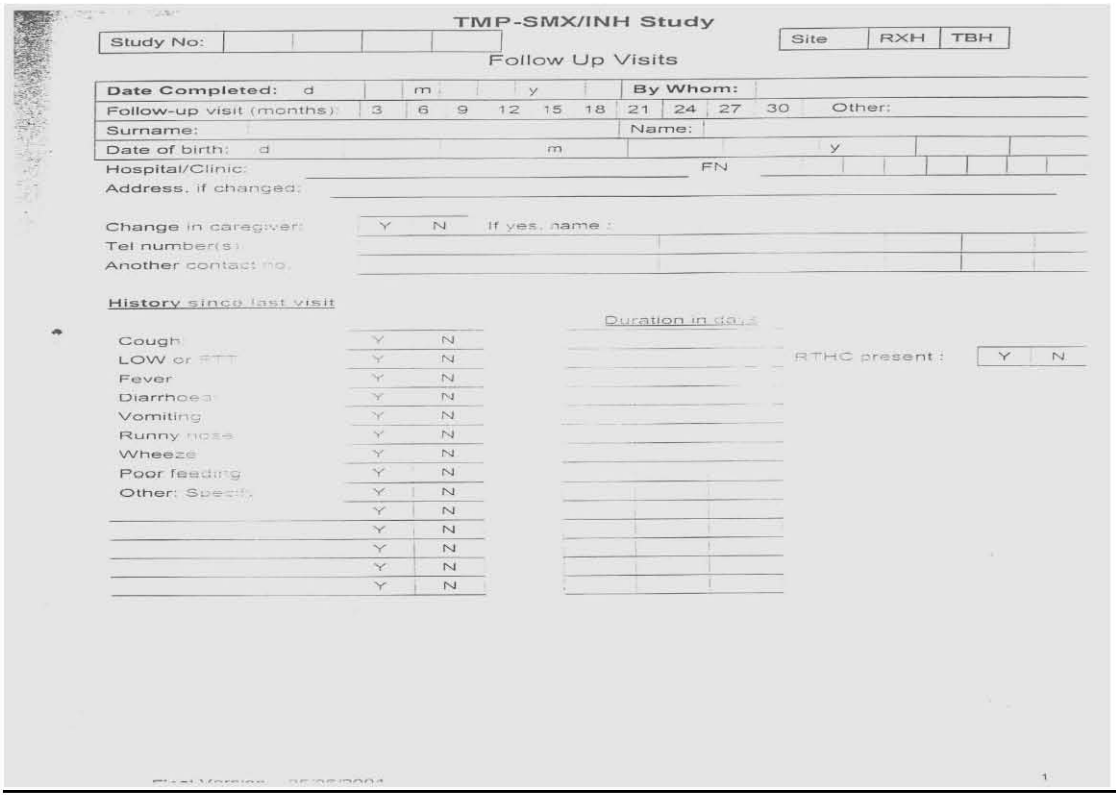
Background
Your child is at risk for developing a chest infection caused by a germ called *Pneumocystis carinii*. This infection may cause a severe chest infection (pneumonia). To prevent this, a medicine called **cotrimoxazole** (or Cozole / Bactrim) should be taken by your child either 3 times a week or everyday. Your child is also at risk for getting tuberculosis (TB) because this is so common in the Cape Town area and because your child is unable to fight off infections very well because of his/her HIV disease. To prevent TB, a medicine called **isoniazid (INH)** may be given.

Purpose of the study
The aims of this study are to
(1) Compare how effective giving cotrimoxazole 3 times a week is with giving it everyday for preventing chest and other infections.
(2) Investigate whether giving INH either 3 times a week or everyday can prevent TB.

Procedures in the study
Your child will be given cotrimoxazole to take either 3 times a week or everyday. Your child will also be given INH as a tablet or a pill that looks like the INH tablet but does not contain INH (placebo) to take either 3 times a week or everyday. Your child will be seen every month at the Infectious Diseases clinic to check how he/ she is doing. At the first visit to the clinic, a blood test will be done to measure your child's ability to fight infection (immune status) and a blood specimen will be stored to measure the amount of HIV virus in your child. A swab from the nose will also be taken and sent to the laboratory where tests will be done to identify the germs living in his/her nose. A nose swab will be done every 6 months on you child to see whether cotrimoxazole or INH change the type of germs in your child's nose. A

1

Appendix 2 Data capture form for each study visit



TMP-SMX/INH Study

Study No: | | | | | Site | RXH | TBH

Follow Up Visits

Date Completed: d | | m | | y | | By Whom: |

Follow-up visit (months): 3 6 9 12 15 18 21 24 27 30 Other:

Surname: Name:

Date of birth: d m y

Hospital/Clinic: FN

Address, if changed:

Change in caregiver: Y N If yes, name :

Tel number(s):

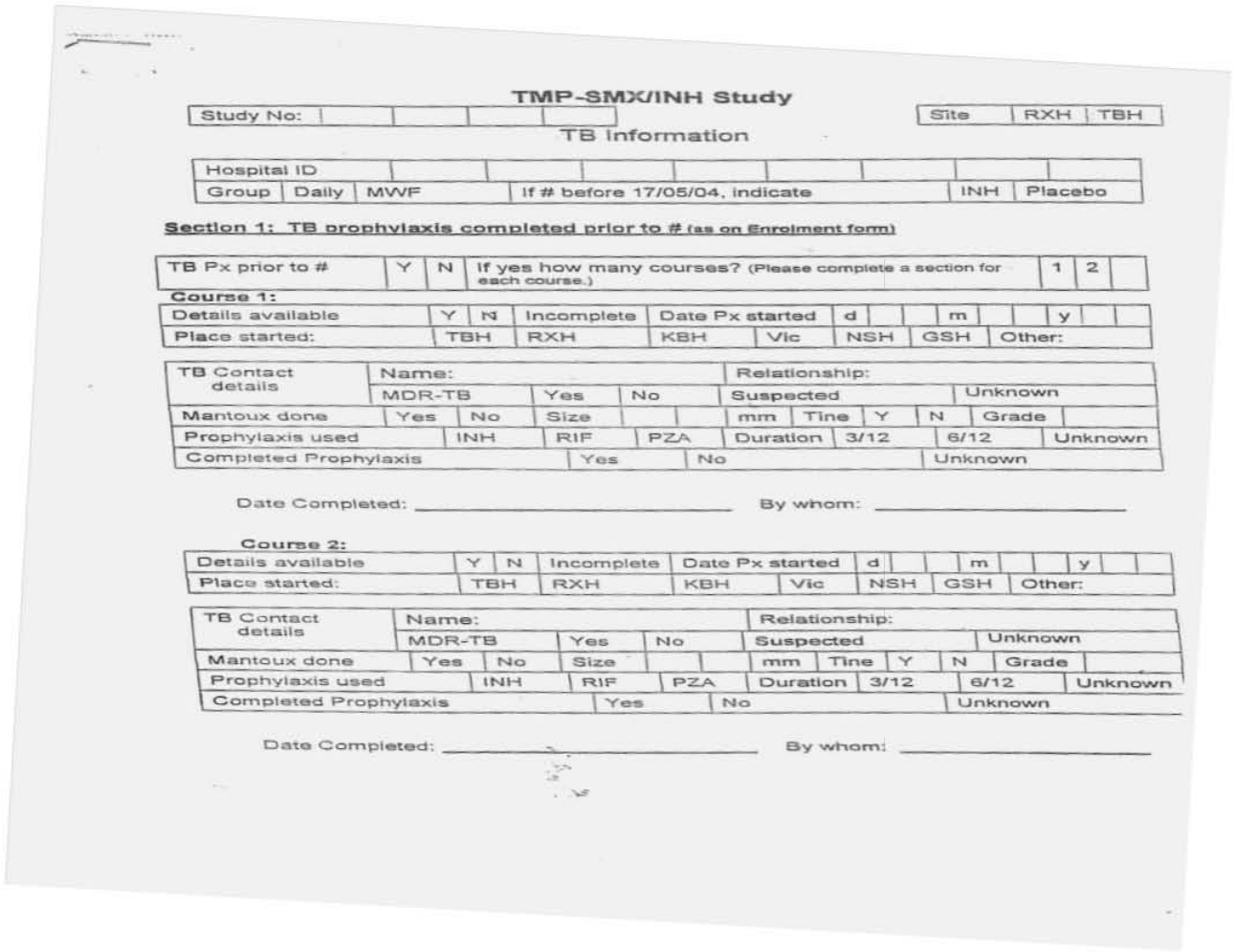
Another contact no.

History since last visit

			Duration in days
Cough	Y	N	
LOW or RTT	Y	N	
Fever	Y	N	
Diarrhoea	Y	N	
Vomiting	Y	N	
Runny nose	Y	N	
Wheeze	Y	N	
Poor feeding	Y	N	
Other: Specify:	Y	N	
	Y	N	
	Y	N	
	Y	N	
	Y	N	
	Y	N	

RTHC present : Y N

Appendix 3 TB data capture form



TMP-SMX/INH Study

Study No:					Site	RXH	TBH

TB Information

Hospital ID									
Group	Daily	MWF	If # before 17/05/04, indicate					INH	Placebo

Section 1: TB prophylaxis completed prior to # (as on Enrolment form)

TB Px prior to #	Y	N	If yes how many courses? (Please complete a section for each course.)	1	2	

Course 1:

Details available	Y	N	Incomplete	Date Px started	d			m			y		
Place started:	TBH	RXH	KBH	Vic	NSH	GSH	Other:						

TB Contact details	Name:				Relationship:							
	MDR-TB	Yes	No		Suspected				Unknown			
Mantoux done	Yes	No	Size			mm	Tine	Y	N	Grade		
Prophylaxis used	INH	RIF	PZA		Duration	3/12		6/12		Unknown		
Completed Prophylaxis	Yes	No			Unknown							

Date Completed: _____ By whom: _____

Course 2:

Details available	Y	N	Incomplete	Date Px started	d			m			y		
Place started:	TBH	RXH	KBH	Vic	NSH	GSH	Other:						

TB Contact details	Name:				Relationship:							
	MDR-TB	Yes	No		Suspected				Unknown			
Mantoux done	Yes	No	Size			mm	Tine	Y	N	Grade		
Prophylaxis used	INH	RIF	PZA		Duration	3/12		6/12		Unknown		
Completed Prophylaxis	Yes	No			Unknown							

Date Completed: _____ By whom: _____

Appendix 4 Approval of protocol

Gray: Confirmation of Approval of Study Proposal

From: Jackie Cogill

To: Diane Gray; Diane Gray

CC: Dianne Pryce; Heather Zar; Lorraine McDonald

Date: Wednesday - April 11, 2012 8:55 AM

Subject: Gray: Confirmation of Approval of Study Proposal

Dear Dr Gray
Herewith attached is the amended Confirmation of Study Proposal. Your plan has been amended from Adult to Paediatric.

Candidature Approval (GRYDIA002)

Degree	MPhil in Pulmonology (Paediatric)
Title	Isoniazid preventive therapy in HIV infected children on antiretroviral therapy living in a high tuberculosis prevalence area: a randomised controlled trial
Department	Child & Adolescent Health
Supervisor	Prof H Zar
Ethics Approval	299/2005

I am pleased to advise that the Chair of the Dissertations Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, PG-Med February 2012.

Yours sincerely

Jackie Cogill