A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL TO DETERMINE THE EFFICACY OF ISONIAZID (INH) IN PREVENTING TUBERCULOSIS DISEASE AND LATENT TUBERCULOSIS INFECTION AMONG INFANTS WITH PERINATAL EXPOSURE TO HIV

An International Multicenter Trial of the International Maternal Pediatric Adolescent AIDS Clinical Trials Group

Sponsored by:

The United States of America National Institute of Allergy and Infectious Diseases and Secure the Future Foundation

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Version 2.0
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GLOSSARY

AER  Adverse Event Report
ALT/SGPT  Amino Alanine Transférase / Serum Glutamate Pyruvate Transaminase
ART/ARV  Antiretroviral therapy/antiretroviral
AST/SGOT  Aspartate Amino Transferase /Serum Glutamic Oxalacetic Transaminase
AUC  Area Under the Curve
BCG  Bacille Calmette-Guerin
CRF  Case Report Form
CSF  Cerebrospinal fluid
DAIDS  Division of AIDS, NIAID
DMC  Data Management Center
DSMB  Data and Safety Monitoring Board
EPTB  Extrapulmonary Tuberculosis
HAART  Highly Active Antiretroviral Therapy
ICH  International Code of Harmonization
INH  Isoniazid
LFT  Liver Function Test
LIP  Lymphocytic interstitial pneumonia
LTBI  Latent tuberculosis infection
MCC  Medicines Control Council, South Africa
* M.t.b.  Mycobacterium tuberculosis
NIAID  National Institute of Allergy and Infectious Diseases
NIH  National Institutes of Health
PACTG  Pediatric AIDS Clinical Trials Group
PACTU  Pediatric AIDS Clinical Trial Unit
PCP  * Pneumocystis jiroveci (previously Pneumocystis carinii) Pneumonia
PID  Patient Identification Number
PK  Pharmacokinetic
PPD  Purified Protein Derivative
PMTCT  Prevention of mother to child transmission
PTB  Pulmonary Tuberculosis
RAB  Regulatory Affairs Branch, DAIDS
RCC  Regulatory Compliance Center
SAE  Serious Adverse Event
SDAC  Statistical Data Analysis Center
SDMC  Statistical and Data Management Center
SID  Study Identification Number
TB  Tuberculosis
TMP/SMX  Trimethoprim-Sulfamethoxazole
TST          Tuberculin Skin Test
USA          United States of America
WHO          World Health Organization

SUMMARY OF CHANGES

Summary of Changes for P1041, A Randomized Double Blind Placebo Controlled Trial to Determine the Efficacy of Isoniazid (INH) in Preventing Tuberculosis Disease and Latent Tuberculosis Infection Among Infants with Perinatal Exposure to HIV, Version 2.0, dated 7/11/07.

All changes in this version appear in boldface type. Editorial changes, corrections of typographical errors, and other changes required to update information that do not affect regulatory issues or patient consent may also be included.

Major changes include the following:

1) P1041 was designed to be done exclusively in South Africa where the risk of developing tuberculosis is the highest and for this reason, the protocol includes some specific information relative only to South Africa. Because accrual of the HIV-infected cohort has not reached expected benchmarks, the team is actively soliciting new sites for enrollment and the expected participation of these sites is reflected throughout specific sections of this version of the protocol.

2) The title of the protocol has changed to allow enrollment at other international sites.

3) The participating site list has been updated to add new sites (Durban, Malawi and Botswana) and to delete the Red Cross Hospital in Cape Town, South Africa, which is no longer participating.

4) The protocol team roster has been updated to add investigators from Durban, Malawi and Botswana.

5) Site numbers (which are undergoing renumbering) have been deleted throughout the protocol and sites are identified only by name.

6) Sites will follow their country specific guidelines for TMP/SMX prophylaxis, use of antiretroviral therapy and TB treatment and this has been noted throughout the protocol.
7) The Schema, and Sections 3.0, 4.6, 8.1 and 9.2 have been revised to state that enrollment of HIV-exposed uninfected infants closed to accrual under Version 1.0 on June 7, 2006.

8) Section 1.6, Background, last paragraph has been added to include information regarding an INH prophylaxis study done in Cape Town, South Africa.

9) Section 1.73, Alternate Prophylaxis with Dapsone and Atovaquone, has been added.

SUMMARY OF CHANGES (Cont.)

10) Section 1.82, Prevention of Mother-to-Child Transmission, has been updated to add breastfeeding recommendations for women in Malawi.

11) Sections 2.1 and 2.2, Primary and Secondary Objectives, and Section 8.0, Statistical Considerations, have been revised to add death as a primary endpoint and to recalculate the statistical power per recommendations made by the Data and Safety Monitoring Board during their reviews in June 2004 and January 2007.

12) Section 4.11, Inclusion Criteria has been revised to clarify the ages that infants can be screened for entry.

13) Section 4.12, Inclusion Criteria, has been clarified to state that a hard copy of the mother’s infection status is not necessary as long as a positive DNA PCR is available for the infant.

14) Section 4.13, Inclusion Criteria has been revised to clarify receipt of BCG vaccine.

15) Section 4.15, Inclusion Criteria changed to the physician’s assessment of neurodevelopment.

16) Section 4.215, Exclusion Criteria has been changed to allow subjects with >/= Grade 3 hemoglobin to enter the study.

17) Section 4.5, Use of Antiretroviral Therapy has been revised to describe the possible impact of antiretroviral therapy on study endpoints.

18) Section 4.6, Enrollment Procedures has been updated to include information on catchment areas in Durban, Malawi and Botswana.

19) Section 4.61, Enrollment Log has been revised to allow permission to collect demographic information on the infant for infants not enrolled in the study.
20) Section 5.32, Distribution of Isoniazid/Placebo has been updated to include dispensing instructions at Durban and Malawi.

21) Section 5.34, Dapsone and Atovaquone have been added to clarify that dapsone and atovaquone will NOT be supplied by the study.

22) Section 6.1, Repeat test for Grade 3 hemoglobin has been clarified.

SUMMARY OF CHANGES (Cont.)

23) Section 6.31, Statement added regarding risk of discontinuing PCP prophylaxis.

24) Section 6.5, TB Exposure and TB Disease Management, has been revised to clarify tracking of suspected TB disease or infection.

25) Section 6.521, has been revised to provide guidance for the treatment of household exposure to TB.

26) Section 6.87, the criterion for treatment discontinuation if a study participant requires INH prophylaxis due to household exposure to TB has been added.

27) Section 7.0, Expedited Adverse Event Reporting, has been updated to include new language but the protocol still follows intensive reporting requirements.

28) Section 10.0, Human Subjects, the statement requiring sites to have a plan that addresses change in guardianship has been added.

29) Section 13.0, Reference 61 has been updated and References 77-84, 95 and 99 have been added.

30) Appendix IA, Schedule of Evaluations for HIV-infected Study Participants, footnote #11 has been revised to clarify the requirements for obtaining the pre-entry DNA PCR sample; footnote #14 has been revised to clarify HIV-1 EIA for subjects who are breastfed; footnote #20 has been revised to clarify timing of pre-entry and entry visits.

31) Appendix IB, Schedule of Evaluations for HIV-uninfected Study Participants, footnote #3 has been added to assess the presence/absence of BCG skin reaction and footnote #17 has been revised to clarify the windows for study visit weeks.
32) Appendix VI, Evaluation of Peripheral Neuropathy, has been revised to clarify the evaluation of peripheral neuropathy for infants 3 months of age.

33) Appendix VII, Virology, Hematology and Immunology Collection and Shipping Instructions, Appendix VIII, Pharmacology Collection and Shipping Instructions, and Appendix IX, Methods for Nasopharyngeal Swabs for the Detection of Streptococcus Pneumonia, have been revised to add the requirement that all specimens must be logged into the LDMS.

34) Appendix XII, Enrollment Log, has been added.

35) Appendix XIII A, Sample Informed Consent:

SUMMARY OF CHANGES (Cont.)

1) Introduction: The last three sentences have been added to obtain permission to use information if the subject does not enroll.

2) Why is This Study Being Done? The last sentence in the first paragraph has been added to state that preliminary data suggest that INH contributes to survival benefit in HIV-infected children; the 2nd sentence in the second paragraph has been added to clarify the chance of receiving INH or placebo; the 3rd sentence in the second paragraph has been added to state that only HIV-infected infants will be enrolled; the last sentence in the last paragraph has been added to state that dapsone or atovaquone will be given if TMP/SMX is not tolerated.

3) Why Would the Doctor Take My Child Off This Study Early? This section has been revised to state that the subject will discontinue therapy if INH is given to prevent TB and the availability of INH has been clarified if the subject ends participation early.

4) What Are the Risks of the Study? The side effects of dapsone and atovaquone have been added.

5) What are the Costs to Me and My Child? The section has been revised to state the study participant will not be charged for INH or TMP/SMX but dapsone and atovaquone are not supplied by the study.
SCHEMA

A RANDOMIZED, DOUBLEBLIND, PLACEBO CONTROLLED TRIAL TO DETERMINE THE EFFICACY OF ISONIAZID (INH) IN PREVENTING TUBERCULOSIS DISEASE AND LATENT TUBERCULOSIS INFECTION AMONG AFRICAN INFANTS WITH PERINATAL EXPOSURE TO HIV

DESIGN: Phase II/III, multicenter, randomized, placebo-controlled, doubleblind

SAMPLE SIZE: Total: 1300 study participants
500 HIV-infected study participants
800 HIV-exposed uninfected study participants (CLOSED TO ACCRUAL under Version 1.0 on June 7, 2006)

POPULATION: Infants born to HIV-infected women will be enrolled between the 91st and 120th day of life.

RANDOMIZATION, STRATIFICATION AND BALANCING: Randomization to INH or INH placebo will be in a 1:1 ratio and will be stratified by the study participant’s HIV infection status:

Stratum I: HIV-infected study participants
Stratum II: HIV-exposed uninfected study participants

Randomization for Population Pharmacokinetic Study:

336 infants will be randomized to undergo population PK sampling at study weeks 0 and 84 or at study weeks 12 and 84 and to blood draw times 1 and 3 hours post-dose or 2 and 4 hours post-dose in a 1:1:1:1 ratio. Half of the study participants will be HIV-infected and half will be HIV-uninfected. Only subjects enrolled at Cape Town and Durban will undergo pk sampling.

REGIMEN: Stratum I: HIV-infected study participants randomized to:

- INH (10 – 20 mg/kg/dose orally, once a day) or placebo beginning any time between the 91st -120th day of life
SCHEMA (Cont.)

Stratum II: HIV-exposed uninfected study participants randomized to:

- INH (10 – 20 mg/kg/dose orally, once a day) or placebo beginning any time between the 91st - 120th day of life.

TMP/SMX Prophylaxis

Regardless of randomization to INH or placebo, all study participants will receive TMP/SMX (5mg/kg dose of the trimethoprim component orally once a day, or according to country specific guidelines) at the time of study enrollment, if not already started at 6-8 weeks of life.

For HIV-infected study participants, TMP/SMX will be given until 12 months of age and will continue after 12 months of age if the study participant meets WHO or country specific guidelines for continuing PCP prophylaxis.

For HIV-exposed, but uninfected study participants, TMP/SMX will continue until a repeat HIV DNA PCR test is performed at 6 months (24 weeks) of age, at which time prophylaxis will be continued if HIV-infected, or if HIV-uninfected but still breastfeeding.

TREATMENT DURATION: Study participants will receive INH or placebo for 96 weeks and will be followed on study for an additional 96 weeks (a total of 192 weeks from the time of study enrollment).

OBJECTIVES:

Primary:

1. To determine whether INH prophylaxis increases TB disease free survival from randomization to 96 weeks for HIV-infected study participants.

2. To determine whether INH prophylaxis increases TB infection free survival from randomization to 96 weeks for perinatally-exposed, HIV-uninfected study participants.
Secondary:

Among both perinatally-exposed HIV-infected and HIV-uninfected study participants:

1. To assess the toxicity and safety of INH prophylaxis.

   SCHEMA (Cont.)

   2. To determine whether INH prophylaxis decreases the incidence of **TB disease from randomization to 96 weeks and 192 weeks.**

   3. To determine whether **INH prophylaxis decreases the incidence of TB infection from randomization to 96 weeks and 192 weeks.**

   4. To determine whether **INH prophylaxis increases TB disease free and TB infection free survival from randomization to 192 weeks.**

   5. To determine whether **96 weeks of INH prophylaxis lowers mortality for 96 weeks and 192 weeks.**

   6. To investigate population PKs of INH among 336 study participants who have been randomized to receive INH/placebo.

Among HIV-infected study participants only:

7. To determine whether **96 weeks of INH prophylaxis increases TB-infection free survival for 96 weeks.**

8. To determine whether **96 weeks of INH prophylaxis slows HIV disease progression or death for 96 weeks and 192 weeks.**

Among perinatally-exposed HIV-uninfected study participants only:

9. To determine whether **96 weeks of INH prophylaxis increases TB-disease free survival for 96 weeks.**

Tertiary Objectives:
1. To describe *Pneumocystis jiroveci*-related morbidity and mortality among all subjects receiving TMP/SMX according to current WHO guidelines.

2. To compare antibiotic susceptibility of *Streptococcus pneumoniae* isolated from nasopharyngeal swabs among those receiving or not receiving TMP/SMX prophylaxis, among all study participants at Johannesburg.

3. To describe the association between INH prophylaxis and development of INH resistance, among all study participants with culture-confirmed TB disease.
1.0 INTRODUCTION

1.1 Tuberculosis Disease Worldwide

In 1997, the World Health Organization (WHO) estimated that almost eight million new cases of tuberculosis (TB) disease occurred throughout the world. Each year, 1.9 million people around the world die of TB, making TB the most common infectious cause of death in the world after human immunodeficiency virus type 1 (HIV-1) infection (1). The incidence of TB in South Africa (243 cases/100,000) is one of highest in the world. The number of cases of TB has been rising in South Africa, from 80,400 in 1990, to 90,200 in 1994, to 105,169 in 1997 (2).

1.2 Surveillance of Tuberculosis in South Africa

According to the South African Tuberculosis Control Program, if an adult is diagnosed with active, smear-positive TB, he or she will be advised to bring any household contacts under five years of age to the clinic for assessment. Any other contacts older than five years of age are told to come to the clinic if they develop TB symptoms. If those who are invited do not visit the facility to be screened for TB, a community health care worker will actively engage in tracing those individuals.

1.3 Co-infection with HIV and Tuberculosis

The prevalence of TB among an HIV-infected population is directly proportional to the TB prevalence in the general community. In South Africa, the prevalence of HIV infection among adults with TB increased from 36% to 65.9% over a period of four years. In Uganda, 73.5% of deaths among TB-infected patients were attributable to HIV infection (3). HIV-infected individuals are at an increased risk of developing TB; immunocompetent adults have a 10% lifetime risk of developing TB after infection with Mycobacterium tuberculosis (M.tb.) compared to an annual risk of 10% among HIV-infected adults (4). A decrease in CD4 lymphocyte counts has been observed in HIV-uninfected patients with TB (4, 5). It is thought that TB can aggravate the immunological deterioration of HIV-infected individuals, and thus hasten HIV disease progression (6, 7). Globally, the case fatality rate from TB is estimated to be 23%, but exceeds 50% in regions such as Africa where the prevalence of HIV infection is extremely high (3).

Among TB patients in South Africa, the HIV seroprevalence rates among those younger than 18 months old and those between 10 and 14 years old were 52% and 14.7%,
respectively (7). It is possible that these variations reflect the increasing HIV seroprevalence rates among young children, as the older children may have been born at a time when overall national (South Africa) seroprevalence rates were lower.

1.4 Pediatric Co-infection with Tuberculosis and HIV in South Africa

Among immunocompetent children infected with *M. tb.*, the risk of developing TB disease and of developing the worst forms of TB disease (miliary and meningeal disease) are much higher than among adults with HIV. In general, the younger the child with TB infection is, the greater the risk of progression to disease. Most cases of pulmonary TB among South African children (both HIV-infected and -uninfected) occur in children under two years of age (median ages of 12 and 14.5 months, respectively) (5).

Although there are no data available regarding the risk of development of TB disease in children co-infected with TB and HIV, it would seem reasonable to assume an even higher risk than that observed in adults, particularly for those children living in countries with a high prevalence of TB. Preliminary data from the University of Zambia Medical School’s primary teaching facility indicate approximately 69% of children hospitalized with clinical TB are co-infected with HIV (8). TB is the fifth most common diagnosis among hospitalized children at this institution, with five percent of children admitted having TB. Similarly, in a study in Cote d’Ivoire, 23.4% of children with TB were co-infected with HIV (9). Another study found that the overall seroprevalence of HIV among children with TB was 37.1% (10). In a study performed in Johannesburg, at a time when approximately 4% of the birth cohort was estimated be to be HIV-infected, 42% of all children in whom TB was diagnosed had concurrent HIV infection (4).

In a study of severe pneumonia conducted in Johannesburg, South Africa, *M. tb.* was isolated in 8% of children hospitalized for acute pneumonia (5). Similarly, high proportions of children with acute pneumonia in whom *M. tb.* was isolated have been described in two other studies defining the etiology of pneumonia in African HIV-infected children (11, 12). The estimated incidence rates of culture-confirmed TB (diagnosed with gastric aspirates) in children aged 2-24 months with acute pneumonia was increased 22.5 fold in HIV-infected (470/105), as compared to HIV-uninfected (65/105) children (5). Considering that the sensitivity of gastric washings in diagnosing TB is only 30-40% (13), the above reported figures probably underestimate the true burden of pulmonary TB accordingly. Hence, as many as one third of children hospitalized with acute pneumonia may in fact have pulmonary TB in a country such as South Africa, with its high prevalence of TB.
Only 25% of the co-infected HIV/TB children in South Africa who are hospitalized have had a known identified household contact with an active case of TB. In the remaining 75%, no known sputum-positive TB contact is identified (5). The South African standard of care for TB involves prophylaxis for children less than 5 years of age when they have had identified household contact with a case of sputum-positive TB (2).

1.41 Pulmonary Tuberculosis

The diagnosis of pulmonary TB (PTB) is based on currently established algorithms that are biased toward investigating children with more chronic symptoms (14, 15, 16). In a study conducted in Johannesburg, *M. tuberculosis* was identified from gastric washings in 41.5% of HIV-infected children with a clinical diagnosis of PTB based on criteria recommended by Migliori and colleagues (14). This algorithm, as well as other algorithms for the diagnosis of PTB in children, has not been scientifically validated (17). The classical criteria of PTB such as chronic cough, weight loss and unremitting fever that are used in the various algorithms are possibly of lesser use in HIV-infected children since many of these criteria could be due to other opportunistic infections, chronic illness such as LIP, or to HIV infection itself (18).

In a study in South Africa, the value of tuberculin skin testing (TST) in assessing HIV-infected children for TB was questionable, with only 25% of HIV-infected children in whom TB was diagnosed having reactions of at least 5 mm (4). Other studies among HIV-infected African children have reported slightly higher (42-50%) rates of reactivity. The results of the studies may have been biased due to retrospective data collection (19, 20).

Recent data suggest that RT23 2 Tuberculin Unit (TU) is adequate to test for TB infection (21, 22, 23). It should be noted that 2 TU (RT23) has been evaluated against PPD-S 5TU and has been found to be biologically equivalent (24).

In HIV-uninfected patients with active TB, 10% to 20% have negative TSTs and 5% to 10% have a negative TST, but have a positive reaction to another antigen. A negative skin test result does not exclude either latent infection or active disease, even in the presence of a reaction to other antigens (19, 20).
Recent reports evaluating the utility and sensitivity of using induced sputum in children as young as two months of age, suggest this method to be superior to gastric washings in the identification of \textit{M.tb} in children with PTB (25).

1.42 Extrapulmonary Tuberculosis

Twenty-five percent of hospitalized TB cases will present with extra pulmonary TB (EPTB), according to an estimate derived from many studies (26, 27). The diagnosis of EPTB is usually more straightforward than that of PTB due to characteristic clinical symptoms (e.g. spinal deformity, scrofula or painful ascites, etc.) and supportive microscopic findings in specimens such as CSF, pleural fluid, ascetic fluid and lymph node aspiration/biopsy.

1.5 Latent Tuberculosis Infection in Adults

A high percentage of the adult population of Sub-Sahara Africa is latently infected with \textit{M.tb}. Previous reports have estimated that by the time an adult acquires HIV infection, half or more of these adults will have been previously infected with \textit{M.tb}. (28, 29). While the life-time risk of a latently infected immunocompetent adult developing TB approaches 10%, the risk of TB reactivation among adults co-infected with both HIV and TB is considerably higher (30). The rate of developing TB among HIV-infected adults has been demonstrated to be 22-25 times that of HIV-uninfected subjects (31, 32).

With regard to the incidence of latent TB infection (LTBI), the annual risk in the Western Cape Town province during a recent, as yet unpublished, annual risk of incidence (ARI) study, was 3% (33). This implies that roughly 3% of the population will be infected each year. It can be concluded that if there is someone infected with HIV in the household, this ARI might be higher than 3% in the target population for this study.

The annual rate of reactivation of TB among HIV-infected adults who are TST+ may be as high as 8-10% per year (34). If approximately 50% of the enrolled HIV-infected mothers in this study have LTBI, and 8-10% of these mothers develop clinical TB each year, then 4-5% of the total number of children initially enrolled will manifest overt TB each year. The risk of infection of the child when exposed to an adult with smear positive TB is \textasciitilde60%, with \textasciitilde40% of these children who are infected by \textit{M.tb} in the first two years of life progressing from infection to disease. For HIV-uninfected children born to HIV-infected mothers, the high prevalence of active TB among their mothers
and other family contacts poses increased risk of infection with subsequent risks of severe disease during infancy and early childhood. If, as has been described previously, TB can act as a co-factor in HIV disease progression (especially in young children), then co-infected children are quite likely to experience greater morbidity and mortality than HIV-uninfected children without concomitant TB infection (35).

1.6 Tuberculosis Chemoprophylaxis

In adults, the use of isoniazid (INH) chemoprophylaxis has been associated with a marked reduction in the risk of TB disease among tuberculin reactors and anergic persons in high-risk populations (36). A case control study in New Mexico found strong evidence (70%) of a decrease in the risk of developing TB disease following use of INH prophylaxis for at least six months (37, 38). In Haitian adults, a combination of INH and pyridoxine (vitamin B6) was almost four times more effective than placebo in preventing the development of TB. The same study noted delayed progression of HIV infection to AIDS or death (39). In several other trials, prophylaxis with various regimens has been shown to be efficacious in preventing TB (40, 41, 42).

The necessary length of prophylaxis and methods of improving adherence remain to be determined. Adherence to TB chemoprophylaxis remains an ongoing problem. Some studies have found that adherence to prophylaxis to be as low as 38% (43). A double blind, controlled, randomized trial in Lusaka, Zambia employed a regimen of twice weekly, rather than daily, INH to enhance adherence. Despite less frequent dosing, INH prophylaxis was still beneficial (42).

The quantity and quality of evidence has led to the formulation of recommendations that patients with latent TB infection should be offered six to 12 months of INH prophylaxis. Prior studies targeting high-risk populations such as HIV-infected intravenous drug abusers, or patients receiving chronic steroid therapy without prior determination of TST reaction by the Mantoux method, have also demonstrated the benefit of INH prophylaxis (40).

A large controlled study by the U.S. Public Health Service established that INH given at a dose of 4-6 mg/kg of body weight significantly reduced the risk of development of TB disease among young children (37). Subsequent to the establishment of 4-6 mg/kg as an effective dose, it was observed that some individuals, including some children, were rapid acetylators of INH. Since INH had been given safely in doses as high as 30 mg/kg, it was recommended that children with latent TB infection be given a daily dose of 10-20 mg/kg, to provide therapeutic blood levels even in the rapid acetylators (44).
In children, delay in initiating INH prophylaxis has been associated with acquisition of TB infection, especially in those cases where an infectious source was identified. A prophylactic INH dose of 9mg/kg body weight was found to be efficacious (27). A similar study in North Carolina assessing opportunities for preventing the transmission of TB to children reaffirmed that adherence to acceptable recommended prophylactic regimens should reduce the childhood TB case rate (26).

In January 2003 a study entitled, “Strategies for prevention of opportunistic infections in HIV-infected South African children: Comparison of two trimethoprim-sulphamethoxazole (TMP-SMX) prophylaxis regimens with and without concomitant isoniazid (INH) – impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis,” was opened in Cape Town, South Africa with the primary objective of evaluating the impact of INH prophylaxis on TB.

A preliminary analysis by the study’s DSMB in October 2003 suggested a survival benefit for INH. A second analysis took place in December 2003, of the first 148 subjects with ≥1 month follow-up. Sixteen of 21 deaths occurred in the placebo arm. This was significant by “intent to treat” (p = 0.004, log-rank test) and “time on treatment” analysis using person-time of exposure (p = 0.0005 – comparison of rates; 2-tailed test). The survival benefit appeared within the first 50 days in all stages of disease severity and in both study centres. Of 14 cases of tuberculosis, 9 were in the placebo and 5 in the INH group (intent to treat analysis: p = 0.077; log-rank). The preliminary data suggested that INH had a significant survival benefit in HIV-infected children (data presented as a late breaker at the International AIDS meeting, Thailand 2004). On final analysis of 263 subjects, mortality remained lower in the INH group (11 [8%] vs. 21 [16%]) in the placebo group; hazard ratio 0.46, 95% confidence interval 0.22 to 0.95; p=0.015 by intent to treat analysis. The incidence of TB was also lower in the INH group (5 cases [3.8%] vs. 13 cases [9.9%]); hazard ratio 0.28, 0.1 to 0.78, p=0.0005 (99).

There are several key differences in this study when compared to P1041. These include the following: (1) Age group targeted for enrollment: 91-120 days of age for P1041 versus 8 weeks to 12 years of age for the other study; P1041 will enroll children with no or mild symptoms as opposed to the other study that enrolled sicker children and those with advanced disease; and (3) P1041 aims to enroll subjects prior to TB exposure (primary prophylaxis) while the other study
recruited a wide range of subjects, many of whom may have been exposed to tuberculosis and may have been infected at time of enrollment (secondary prophylaxis) as well as one-third of whom had been previously treated for TB. None of the children in P1041 will have received TB treatment prior to enrollment.

1.61 Use of Bacille Calmette-Guerin Vaccine for Tuberculosis Prevention

In developing countries, control measures for TB have focused on Bacille Calmette-Guerin (BCG) vaccine as well as case detection and treatment (45). The efficacy of BCG vaccine in children is low, and recent studies have demonstrated that receipt of BCG in childhood does not prevent TB in adulthood. In fact, BCG vaccine is only protective against miliary TB and TB meningitis. In addition, a recent decision analysis found that preventive therapy would be more effective for TB control than BCG among HIV-infected individuals (46).

1.62 Isoniazid Pharmacokinetics Among South African Children

Isoniazid was first studied in U.S. children between 1955-1960 in the U.S. Public Health Service Tuberculosis Prophylaxis Trial. The objective of the trial was to test whether INH treatment of primary TB disease could prevent tuberculosis meningitis and other post primary complications (37, 47). The basis for the trial was the observation by Lincoln and others that meningitis did not develop among children whose treatment for other forms of TB included INH (48). The study was limited to children with asymptomatic primary TB. In children <3 years of age, this was defined as a PPD (5 TU) reaction of >5 mm of induration while children older than 3 years also had to have roentgen graphic evidence of primary TB. If in the opinion of the site investigator, a child needed additional treatment, the child was excluded.

The trial randomized 2750 children to receive either INH at a dose of 4-6 mg/kg by mouth once daily or placebo. A total of 1394 children were randomized to the INH arm and 1356 to placebo. Randomization by age and treatment assignment was as follows:

<table>
<thead>
<tr>
<th>Age</th>
<th>INH</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (Percentage) of Children</td>
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Results showed that 153 had unfavorable changes during the year of treatment; of these, 88 were pulmonary: 30 in the INH group and 46 in the placebo arm. The vast majority of these changes were increases in the size of parenchymal lesions. Fifty-seven developed extra pulmonary changes. Of these, only 33 were considered definite complications. Two of these children (2/33) had received INH while 31 had been randomized to placebo.

This study conclusively demonstrated the efficacy of INH in preventing the development of severe TB disease in young children at high risk. It formed the basis for the initial recommendation by the USPHS for universal administration of INH at a dose of 5 mg/kg to children with latent TB infection (LTBI).

The 10 mg/kg dose of INH was found to be effective in preventing TB disease when studied in school aged children in San Francisco from 1958-1966. One case developed among 2910 children (0.34 cases/1000) and adolescents who received INH while 25 cases developed among those who did not receive prophylaxis (20.9/1000) (50).

A number of smaller studies through the years have reached conclusions about various vehicles that INH can be mixed with at the time of administration, but no large scale systematic PK trials have been done.

These data demonstrate the efficacy of INH at doses of 5 mg/kg and 10 mg/kg. However, the PKs of the drug at the 10 mg/kg dose administered as a crushed pill in a population of this age (<2 years of age) has not been established even though it is standard practice throughout the world. As this group of young children benefits perhaps more from INH therapy than any other, it remains important to establish the PK parameters of INH in this group.

INH is currently approved and available from the Department of Health in South Africa for use as short-term prophylaxis (6-9 months) for children exposed to TB. It is administered free of charge to children and their families.
1.63 Common Side Effects of Isoniazid

The most frequent adverse events observed with INH are peripheral neuropathy and hepatotoxicity (see package insert).

1.631 Peripheral Neuropathy

Peripheral neuropathy, while unusual, is the most commonly reported INH toxicity, and occurs most often in malnourished individuals and those who are 'slow acetylators'. A Japanese study found slow acetylator genotypes for N-acetyltransferase in all individuals with INH neuropathy (51). This finding confirmed that individuals with slow acetylator genotypes may develop neuropathy after the administration of INH, but the relevance of acetylator phenotype to other side effects of INH is not well defined (52). As a result, it is recommended that prophylaxis with pyridoxine as supplementation during a short-course of INH for TB is not necessary, but should be implemented when high doses of INH [>20 mg/kg/dose] are prescribed for adults and children over 20 kg of body weight who are malnourished or who are slow acetylators (49).

Increased toxicity due to a long course of INH prophylaxis would not be expected. Toxicity due to INH appears to be either dose-related or idiosyncratic, with virtually all toxicities occurring in the first two to four months of administration of the drug (53, 54, 55). Peripheral neuropathy can develop somewhat later during therapy in adults. However, it is rare even in malnourished children and dietary pyridoxine should be adequate to prevent nervous system complications in the study population (56).

1.632 Hepatotoxicity and Hepatitis

In a recent review of hepatotoxicity and transaminase measurements, data from five studies of children receiving INH prophylaxis revealed transient elevations in transaminases in up to 13.6% of children, depending on the study (53). Although 75/955 (7.9%), of children in one study had transient increases in their transaminase levels, INH was stopped in only four children. Among 4473 children receiving INH, 58 (1.3%) had liver function tests drawn because of symptoms suggestive
of clinical hepatitis. Three of the 58 had abnormal results. One of these three had fulminant liver failure. INH administration was discontinued in the remaining two children (55).

There are no data to indicate that hepatitis C is a problem in pediatric populations in sub-Saharan Africa (55, 57, 58).

1.64 Development of INH Resistance in South Africa

Rates of resistance to anti-TB drugs in South Africa fell significantly from 1965 to 1988. For INH, the rate of primary resistance fell from 14% in 1965-70 to 9.4% in 1987-88 (59). This rate is similar to the 7.2% INH primary resistance rate seen in the U.S. between 1994-97 (60).

In the most comprehensive evaluation of drug resistance in the Western Cape Province, the rate of primary INH resistance among adults was 3.9% and that of multiple drug resistance 1.1% (59).

In a prospective study of all pediatric culture results between 1994 and 1998 at Tygerberg hospital, resistance rates of 6% (INH resistance) and 1% (multiple drug resistance) were observed. **Isoniazid resistance increased significantly from 6.9% (21/306 children) in the previous survey to 12.8% (40/313 children) in the recent survey (odds ratio (OR) 1.99, 95% confidence interval (CI) 1.11-3.59). Resistance to isoniazid and rifampicin (multidrug resistance) did not increase significantly between the surveys (7/306 in previous survey vs 17/313 in recent survey; OR 2.45, 95% CI 0.94-6.62) (61).**

1.7 Pneumocystis jiroveci Pneumonia

*Pneumocystis jiroveci* pneumonia was previously known as *Pneumocystis carinii* pneumonia (62). Although the causative agent has been renamed to *jiroveci*, this type of pneumonia is still commonly abbreviated as PCP. Early during the HIV epidemic, *P. jiroveci* was recognized as being an important pathogen causing pneumonia in HIV-infected children in the developed world, yet its importance has only recently been quantified in sub-Saharan Africa. PCP accounts for 10-45% of pneumonia among hospitalized HIV-infected children (11, 12, 63-66). It has been estimated that approximately 40% of HIV-infected children in developed countries develop PCP at some point, with most cases occurring before six months of age (67). Differences in the
proportion of children in whom *P. jiroveci* was isolated in the various studies, such as 10% in the study from Cape Town versus 45% in the study from Johannesburg, could be attributable to differences in selection criteria for study enrollment, as well as differences in PCP diagnostic techniques (13, 63). Obtaining samples using both the induced sputum and nasopharyngeal aspirate techniques have been shown to have a better yield (sensitivity 75% and specificity 80%) than when either method is used independently (63).

### 1.71 Prophylaxis with Trimethoprim/Sulfamethoxazole in South Africa

Trimethoprim/Sulfamethoxazole (TMP/SMX) prophylaxis among HIV-infected children has been shown to decrease PCP-related hospitalization, HIV disease progression, and mortality (67, 68, 69). Consequently, it is recommended that TMP/SMX prophylaxis be standard of care for all infants born to HIV-infected mothers at least until one year of age, unless HIV infection can be definitively excluded. Subsequent prophylaxis (for South African HIV-infected children older than one year of age) is based on the immunologic status (i.e., prophylaxis administered if the CD4 percent is <15%) (69). In Malawi, all HIV-exposed infants are given TMP/SMX until confirmed HIV negative at 18 months of age and all HIV-infected infants continue TMP/SMX regardless of CD4 percent. Despite these recommendations, there is a lack of logistical support for the implementation of TMP/SMX prophylaxis in developing countries, even where antenatal HIV testing of the mother is widespread, as it is in Soweto, Gauteng province and the Western Cape province of South Africa.

The effectiveness of TMP/SMX prophylaxis for prevention of PCP appears to be suboptimal among South African HIV-infected children. In a recent study, the effectiveness of TMP/SMX among HIV-infected children hospitalized for severe pneumonia was only 36% (95%CI - 15.4%, 64.5%) (70). Concurrent *P. jiroveci* infection was observed in 6 of 18, 11 of 26, and 4 of 6 HIV-infected subjects who had bacteremia, a respiratory virus isolated, or *Mycobacterium* species isolated, respectively (63). It is unclear as to whether this is due to poor adherence to prophylaxis or whether there may be strains of *P. jiroveci* that have reduced susceptibility to sulfa drugs (71, 72). Consequently, it is important to elucidate the effectiveness of TMP/SMX prophylaxis in an area such as the target study area and to define the reasons for failure of prophylaxis when this occurs. TMP/SMX prophylaxis at the time when HIV-exposed children present for immunizations, has been shown to be
efficacious in reducing clinical pneumonia, despite these children not having been investigated for PCP (73).

The burden of PCP is greatest in African children under 6 months of age (11, 12, 63, 66). Success in reducing the overall burden of PCP and pneumonia, and possibly improving the quality of life, while delaying the progression of HIV infection, would require prophylaxis to be started as early as six weeks of age. This could be achieved by integrating TMP/SMX prophylaxis into the immunization program of the infant.

1.72 Trimethoprim/Sulfamethoxazole Resistance

A persistent concern regarding TMP/SMX prophylaxis is the potential deleterious impact that widespread TMP/SMX prophylaxis could have on the emergence of drug resistance of pathogens other than *P. jirovei*. There are studies that suggest that the receipt of TMP/SMX prophylaxis is associated with a reduced incidence of bacterial pneumonia including that due to *S. pneumoniae* (74). Yet, *S. pneumoniae* isolated from children who developed invasive pneumococcal disease while receiving TMP/SMX prophylaxis are more likely to be resistant to antimicrobials. Similar results have been reported for *S. pneumoniae* nasopharyngeal isolates among HIV-infected children receiving TMP/SMX prophylaxis in the US (75). In a recent study performed among South African children, 60% of infants who had not received a pneumococcal conjugate vaccine were found to be colonized by *S. pneumoniae* at nine months of age. These *S. pneumonia* isolates had reduced susceptibility to at least one class of antibiotics. Furthermore, 41% and 35% of isolates showed reduced susceptibility to penicillin and TMP/SMX, respectively. These data may be used as a crude comparator in defining the impact that TMP/SMX prophylaxis could have on antibiotic susceptibility (76).

1.73 Alternate Prophylaxis with Dapsone and Atovaquone

Generally, there are limited data and/or recommendations regarding how to manage children and infants who require prophylaxis against PCP and in whom TMP-SMX is contraindicated. In South Africa, there are also no guidelines as to how to manage such children. The alternate available options against PCP prophylaxis are dapsone (2 mg/kg/day or 4mg/kg per week, maximum 100 mg (77) and atovaquone, but atovaquone is not available in Malawi. An alternate option, such as
aerosolized pentamidine, for which there are also only limited data in children, is unavailable as an option for prophylaxis in South Africa and in Malawi.

**Dapsone:** There have been a few favorable reports on the potential usefulness of dapsone as PCP prophylaxis in HIV-infected children (78, 79). McIntosh et al. found that PCP occurred most frequently with the daily dapsone dose of 1-mg/kg regimen (22.0 cases/100 patient years), least frequently with the daily 2-mg/kg regimen (0 case/100 patient years) and at intermediate frequency with the weekly regimen (9.5 cases/100 patient years). A study on dapsone clinical characteristics on oral clearance (CL/F) found that the CL/F was significantly increased by 50% in children taking rifabutin, by 39% in black children, and by 38% in children younger than 2 years old. Although no significant correlations were found between any dapsone exposure parameter and markers of toxicity, increased AUC was associated with a decreased risk of *Pneumocystis carinii* pneumonia (PCP). Although the study advocated that monitoring of serum dapsone levels may be needed to optimize management of PCP prophylaxis with dapsone (80), this is not feasible in South Africa. An earlier study found that both short and long term hematologic toxicities were marginally greater in children receiving the daily 2 mg/kg compared with the weekly regimen (79). Allergic skin rashes were similar in children receiving the daily and weekly regimens (17% in both) and were not associated with prior history of rash with TMP/SMX. Therefore, although a weekly dapsone regimen of 4 mg/kg produced less hematologic toxicity than a daily regimen of 2 mg/kg, this advantage was offset by a trend toward higher breakthrough rates of PCP. Side effects of dapsone include nausea/vomiting, rash and oral lesions (81) and methemoglobinemia which is usually asymptomatic. Dapsone should be stopped if the metHb is >10-15%. Other sulfone syndrome related adverse events include fever, hemolytic anemia, atypical lymphocytes and liver injury (82).

**Atovaquone:** Data on the use of atovaquone as PCP prophylaxis in children is even more limited than that for dapsone. In a study by Hughes et al, the oral suspension of atovaquone was found to be safe and well tolerated in children. A single daily dose of 30 mg/kg provides bioavailability considered adequate for therapy of *Pneumocystis carinii*
pneumonia, but infants between 3 and 24 months of age may require a dosage of 45 mg/kg/day (83). Side effects of atovaquone include rash, gastrointestinal symptoms, headache and insomnia. As there are no other available options for the management of PCP prophylaxis in the absence of TMP-SMX and dapsone, the study team proposes the use of atovaquone as a third-line agent. Although studied in another context, recent study showed that atovaquone-azithromycin was as effective as TMP-SMX for the prevention of serious bacterial infections and similarly tolerated in HIV-infected children (84).
1.8 Study Rationale

1.81 Relationship of High Maternal HIV Seroprevalence and Risk of TB Exposure and Disease in South African Children

Both TB and HIV are major public health problems in South Africa. A relatively large portion of the adult population of South Africa has pulmonary TB. The seroprevalence of HIV among pregnant women in South Africa is high, 31% in Soweto and 12.1% in the Western Cape, making them a population at high risk of exposure to TB. The high seroprevalence of HIV and high risk of exposure to TB among pregnant women in this region increases the risk of TB disease in children, especially during the first two years of life, since these children have an increased risk of TB disease, especially disseminated forms, following infection. In order to investigate how to minimize the risk of exposure to TB infection and disease, this trial proposes to determine the efficacy of INH prophylaxis in decreasing TB disease among HIV-infected children and in decreasing TB infection among HIV-exposed, uninfected children.

1.82 Prevention of Mother-to-Child Transmission of HIV

Although effective interventions to prevent mother-to-child transmission (MTCT) of HIV during the ante partum and intrapartum periods have been developed, significant obstacles to implementation still remain (85). The standard of care in South Africa is to offer medications including (but not limited to) zidovudine and nevirapine to mothers in the hopes of preventing MTCT of HIV. HIV-infected mothers are counseled to avoid breastfeeding, with the provision of formula by the South African government. The current standard of care at the Cape Town and Johannesburg sites is to offer subsidies or free formula to HIV-infected women. This formula will be available to all children enrolled into P1041.

Breastfeeding among HIV-infected women in South Africa and Botswana is uncommon, due to the available free infant formula given by the South African Government. But if the mother prefers to breast feed then she is allowed to do so. In Malawi, women are recommended to exclusively breastfeed for 6 months with rapid weaning if alternative food sources are available.
according to WHO guidelines for resource poor settings. Data will be collected regarding INH prophylaxis among both breast and bottle fed children.

Study participants will enter this study at four months of age, most likely after the time period associated with exclusive breast-feeding, and thus will not meet the criteria for pyridoxine (Vitamin B6) supplementation. For children who are exclusively breast fed after four months, pyridoxine will be offered as a prescription by their primary care provider.

1.83 Risks of Tuberculosis Infection and Disease and Period of Isoniazid Prophylaxis Administration

HIV infection increases the risk of development of TB disease and TB appears to be a co-factor in HIV progression. Therefore, the risk of TB disease would be even greater in young children co-infected with HIV and M. tb. For perinatally-exposed HIV-uninfected children, the high prevalence of TB disease among family contacts poses an increased risk of TB infection (even without being HIV-infected themselves), with subsequent risks of severe disease in infancy and early childhood and lifelong risks of the reactivation of TB disease.

The administration of INH prophylaxis to HIV-exposed children with a significant likelihood of exposure to TB could represent an effective means of preventing TB infection and/or disease (among all children). Among HIV-infected children, INH prophylaxis can provide an effective means of preventing HIV disease progression. If this trial demonstrates the efficacy of prophylaxis of TB infection among HIV-uninfected children and/or TB disease among HIV-infected children with an inexpensive medication, such as INH, these results would provide the basis for an important public health intervention.

INH prophylaxis for two years is being evaluated in this trial because the first two years of life are considered to be the period of greatest risk for the more severe, disseminated forms of TB (e.g., meningitis and/or miliary disease, etc.), and the risk of progressing from TB infection to disease is greatest (~42%) during this period. INH prophylaxis is not being initiated until three months of age so as not to interfere with response to the live attenuated BCG vaccine administered during the neonatal period.

Additional objectives of this study are to determine whether INH prophylaxis is associated with lower mortality during and after receipt of study drug, and a
lower incidence of TB infection and disease after the two-year period of prophylaxis. It is important to determine whether any efficacy observed with INH prophylaxis is durable, or whether TB infection and disease occur at high rates once prophylaxis has ended. If the latter occurs, it may be that INH prophylaxis for two years merely shifts the burden of TB infection to older children. Such results could affect the ultimate utility and cost-effectiveness of such an approach.

1.84 Use of INH Placebo

This trial will administer study treatment in a double blind placebo-controlled manner (1:1). Neither the family nor the medical care team will be able to identify who is receiving INH. All children who develop either PPD conversion (≥5mm for HIV-infected, ≥10 mm for HIV-uninfected children), or TB disease will be treated as per the National Tuberculosis Control Programme of South Africa, Department of Health (2) or country specific Tuberculosis Control Program. Placebo will not be given in place of a proven effective TB prophylaxis or treatment regimen, and all children who develop TB infection or disease will discontinue INH/placebo, will continue to be followed on study, and will be managed as per local guidelines.

1.85 Assessment of the Efficacy of INH and TMP/SMX Effectiveness as Prophylactic Agents against M. tb and P. jiroveci, respectively, in Relation to the Incidence of Resistance

Susceptibility of subjects’ M. tb. isolates will be determined to assess any association between receipt of INH prophylaxis and incidence of INH resistance.

In South Africa, a significant proportion of pneumococcal isolates from sterile sites have been found to be resistant to TMP/SMX among children who had been exposed to TMP/SMX prophylaxis, compared to those isolated from children who had not been on prophylaxis at the time of their invasive pneumococcal disease episode (86). Receipt of TMP/SMX prophylaxis in HIV-infected children also has been found to have a greater association with the emergence of multiple drug resistant strains of pneumococci. Rates of antimicrobial resistance to TMP/SMX and other antibiotics, already known to be high in this region, will be evaluated in relation to prolonged TMP/SMX prophylaxis susceptibility profile of pneumococci isolated from the nasopharynx
of children receiving TMP/SMX prophylaxis. This evaluation will be done exclusively at Johannesburg. TMP/SMX will be provided to study subjects in accordance with WHO (http://www.who.int/3by5/mediacentre/en/Cotrimstatement.pdf) or country specific guidelines.

Based on these observations, although it is apparent that there is a high degree of morbidity and mortality associated with PCP in African HIV-infected children, it is not clear why TMP/SMX has not been effective in reducing PCP among HIV-infected children (66).

1.86 Assessment of the Safety and Pharmacokinetics of INH

Since there are few available data regarding INH prophylaxis among young infants, especially HIV-infected infants receiving TMP/SMX prophylaxis, the safety and pharmacokinetics of INH will be evaluated. Frequent monitoring for adverse events will be done for all HIV-infected subjects, most or all of who will be receiving TMP/SMX.

1.87 Determination of INH Metabolism in Young Children and its Relation to the Efficacy of INH

As there are few data describing expected ranges of INH pharmacokinetic parameters in children less than 3 months of age, target ranges (absorption rate constant, clearance, and volume of distribution) have been extrapolated from available pediatric and adult data. This extrapolation has not been shown to be accurate for other hepatically-metabolized agents such as protease inhibitors and non-nucleoside reverse transcriptase inhibitors. In one published intensive PK study of 94 Indian children 0-13 years (age distribution not reported), the Cmax ranged between 4.4-8.2 mg/L at a dose of 10 mg/kg/day (87), which is appropriate based on current practice. In patients with TB disease who qualify for therapeutic drug monitoring, the extrapolated peak INH concentration (Cmax) is the parameter of interest. The expected and targeted adult peak INH concentration is 3-5 mg/L at a dose of 300 mg/day (approximately 5 mg/kg) (88). If the concentration falls below this range, dose adjustments are recommended. Since INH has a wide therapeutic index, an INH dose sufficient to achieve therapeutic plasma levels among fast acetylators (10-20 mg/kg) will be administered to all children.
The main route of INH metabolism (to an inactive form) is through acetylation by hepatic N-acetyl acetyltransferase-2 (NAT-2). The rate of this acetylation was first described as a bimodal distribution, but more recent evidence from adult studies indicates a trimodal distribution among the general population. In addition, concordance between acetylator phenotype and NAT-2 genotype has been demonstrated in adult studies of INH PKs (89). Maturation of the hepatic enzyme system occurs within the first few years of life and depends upon the enzyme activity in question. The average age of NAT-2 maturation is not currently known. However, it is well known that the PK of several drugs, particularly those metabolized by the liver, varies with age of the child. The acetylation rate of INH was evaluated in sixty-one children age 4 days to 17 years. INH clearance significantly increased with age, and the youngest fast acetylator observed was 54 days old. Intermediate acetylators (heterozygotes) were not strongly distinguishable from fast acetylators. Based on the data, it was concluded that postnatal maturation of INH acetylation occurs at some time during the first four years of life, but genotypic confirmation of this is needed (90). In another recent study, Schaaf and colleagues presented data from an intensive PK evaluation of INH in 64 TB-infected children (median age 3.75 years). The data showed a marked difference in the rate of metabolism among slow and fast acetylators, but there was no clear distinction between intermediate and fast acetylators (91). Genotypic analysis was not reported in this study. Based on these data, whether or not a trimodal distribution can be demonstrated in the pediatric population remains uncertain. An attempt to correlate the rate of INH clearance (or the acetylation phenotype) to NAT-2 genotype may provide insight regarding the average age of NAT-2 maturation in children.

Population pharmacokinetics has been chosen as the method for the evaluation of INH concentration variability among the study participants. This method seeks to identify the measurable physiologic factors that alter dose-concentration relationships and the extent of these alterations, so that a dosage can be appropriately modified if a clinically significant shift is identified. Population pharmacokinetics is also employed for the evaluation of demographic (race), pathophysiologic (HIV serostatus), or therapeutic (use of TMP/SMX prophylaxis for PCP) features that can regularly alter dose-concentration relationships.

Through the use of population PK modeling, we will estimate INH PK parameters in this population including the absorption rate constant (Ka),
apparent total body clearance, and apparent volume of distribution in study participants 3-24 months of age.

1.88 Monitoring of Adherence

There are five potential reasons for failure of INH prophylaxis in P1041: primary INH resistance, lack of (or poor) adherence, malabsorption of INH, rapid metabolism (acetylator status), and advanced HIV disease.

To evaluate adherence, two previously developed PACTG adherence modules will be administered through a standardized scripted interview at 12-week intervals beginning at the study week 12 visit (92). Self-reported adherence, obtained by a questionnaire administered in a standardized manner, has been shown to be a useful measure of medication taking behavior that predicts virologic response to antiretroviral therapy (92, 93). Results obtained by self-report correlate with other measures of adherence such as pill counts and electronic monitoring (93, 94).

Adherence Module 1 will collect information on the ability of the caregiver to identify the medications (INH and trimethoprim-sulfamethoxazole) and the number of doses missed in the preceding three months. Adherence Module 2 will collect information on difficulties encountered with the administration of the medications. In addition, problems with adherence/toxicity will be queried at each monthly medication visit.

The modules will be specifically modified for this study. All infants will be receiving INH/placebo and TMP/SMX at least until HIV infection status is determined. These will be listed on the Medication List Table prior to each visit. Other prescribed medications and any alternative medication will also be listed.

2.0 STUDY OBJECTIVES

2.1 Primary

2.11 To determine whether INH prophylaxis increases TB disease free survival from randomization to 96 weeks for HIV-infected study participants.
2.12 To determine whether INH prophylaxis increases TB infection free survival from randomization to 96 weeks for perinatally-exposed, HIV-uninfected study participants.

2.2 Secondary

Among both perinatally exposed HIV-infected and HIV-uninfected study participants:

2.21 To assess the toxicity and safety of INH prophylaxis.

2.22 To determine whether INH prophylaxis decreases the incidence of TB disease from randomization to 96 weeks and 192 weeks.

2.23 To determine whether INH prophylaxis decreases the incidence of TB infection from randomization to 96 weeks and 192 weeks.

2.24 To determine whether INH prophylaxis increases TB disease free and TB infection free survival from randomization to 192 weeks.

2.25 To determine whether 96 weeks of INH prophylaxis lowers mortality for 96 weeks and 192 weeks.

2.26 To investigate population PKs of INH among 336 study participants who have been randomized to receive INH/placebo.

Among HIV-infected study participants only:

2.27 To determine whether 96 weeks of INH prophylaxis increases TB-infection free survival for 96 weeks.

2.28 To determine whether 96 weeks INH prophylaxis slows HIV disease progression or death for 96 weeks and 192 weeks.

Among perinatally-exposed HIV-uninfected study participants only:

2.29 To determine whether 96 weeks of INH prophylaxis increases TB-disease free survival for 96 weeks.
2.3 Tertiary

2.31 To describe *Pneumocystis jiroveci*-related morbidity and mortality among all subjects receiving TMP/SMX according to WHO guidelines.

2.32 To compare antibiotic susceptibility of *Streptococcus pneumoniae* isolated from nasopharyngeal swabs among those receiving or not receiving TMP/SMX prophylaxis, among all study participants at Johannesburg.

2.33 To describe the association between INH prophylaxis and development of INH resistance, among all study participants with culture-confirmed TB disease.

3.0 STUDY DESIGN

P1041 is a Phase II/III randomized, double blinded, placebo-controlled trial comparing INH to placebo in 1,300 children perinatally exposed to HIV, with stratification according to HIV infection status and site of enrollment. It is a multicenter study being conducted at international sites only.

Five hundred HIV-infected study participants (425 evaluable study participants) will be randomized in a 1:1 ratio to receive either INH or INH placebo. Eight hundred HIV-exposed, but uninfected, study participants (680 evaluable study participants) also will be randomized in a 1:1 ratio to receive either INH or INH placebo. Enrollment of exposed, uninfected study participants closed to accrual on June 7, 2006 under Version 1.0 of the protocol. INH prophylaxis will be initiated between 91 and 120 days of life at a dose of 10-20 mg/kg once a day. Study participants will receive INH for two years (96 weeks) post-randomization and then will be observed for an additional two years (96 weeks) for a total of 192 weeks on study.

All HIV-infected study participants will receive TMP/SMX as first line agent for PCP prophylaxis during their first year of life. After the first year of life and while on study, PCP prophylaxis will be continued according to country specific guidelines.

Based on the potential risk of postnatal HIV transmission from the mother to child, particularly if there is ongoing exposure to breast milk, all HIV-exposed, uninfected children will also be continued on TMP/SMX prophylaxis at least until a repeat HIV DNA PCR test is performed at 6 months (24 weeks) of age in these children. Following confirmation of the child being HIV-1 uninfected at 6 months of age, TMP/SMX prophylaxis will be stopped in uninfected children that are not being breastfed and continued among those still being exposed to breast-feeding.
TMP/SMX prophylaxis will be continued in the latter group of children until HIV infection status has been determined based on EIA at the 60-week study visit. TMP/SMX prophylaxis will be continued among children in whom the HIV DNA PCR test is reactive at 6 months (24 weeks) of age, and these children will subsequently be managed the same as other HIV-infected children until infection status is resolved.

At Cape Town and Durban only, a subset of the 336 study participants will participate in population pharmacokinetics and will have an INH concentration assessment at either study weeks 0 and 84 or study weeks 12 and 84 at blood draw times 1 and 3 hours post-dose or 2 and 4 hours post-dose in a 1:1:1:1 ratio. Samples from 168 study participants who have received INH will be used to estimate INH population PK parameters. The systemic exposure to INH will be compared to existing adult and pediatric mean values to evaluate whether the INH dose (10-20 mg/kg) produces similar systemic exposure within this patient population.

At Johannesburg only, all study participants will have nasopharyngeal swabs obtained at specified intervals to determine the incidence of antibiotic susceptible *Streptococcus pneumonia*.

The schedule of laboratory and clinical evaluations for this study is outlined in Appendix IA, Schedule of Evaluations for HIV-Infected Study Participants and Appendix IB, Schedule of Evaluations for HIV-Uninfected Study Participants.

4.0 SELECTION AND ENROLLMENT OF STUDY PARTICIPANTS

4.1 Inclusion Criteria

4.11 Age ≥ 91 days to ≤ 120 days. Infants can be screened for entry at > 28 to < 120 days of age, but may not begin the study until they are at least ≥ 91days of age (at least 90 days after receipt of Bacille Calmette-Guerin (BCG) vaccine)

4.12 Documented maternal HIV infection defined as two positive test results which can be any combination of the following (hard copy of positive result is not necessary provided a positive DNA PCR is available for the infant):

- HIV antibody (ELISA, with confirmation by Western Blot);
- HIV DNA PCR;
- HIV RNA PCR > 10,000 copies/ml;
- Neutralizable HIV p24 antigen;
- HIV ELISA rapid test assay (WHO guidelines);
4.13 Documented receipt of BCG vaccine up to and including the 30th day of life and **not less than 90 days prior to randomization into the study**.

4.14 Anticipated ability to complete all study evaluations.

4.15 **Physician assessment of age-appropriate neurodevelopment in which the chronological age is corrected for gestational age for prematurely born infants**

4.16 Parent or legal guardian able and willing to provide signed informed consent and anticipate residence in the area for at least four years.

4.17 For inclusion in the HIV-infected stratum, the infant must have a positive HIV-1 DNA PCR; for inclusion in the HIV-uninfected stratum, the infant must have a negative HIV-1 DNA PCR performed at ≥ 4 weeks of age.

4.2 **Exclusion Criteria**

4.21 Previous diagnosis of TB infection, TB disease or current treatment for TB infection or TB disease.

4.22 Previous receipt of INH.

4.23 Contact with a known AFB sputum smear or culture-positive case of TB before enrollment. Infants born to mothers who have not completed TB prophylaxis for maternal infection or TB treatment for maternal disease prior to labor and delivery are not eligible.

4.24 Current acute or recurrent (≥ 3) episodes of lower respiratory tract disease.

4.25 Chronic persistent diarrhea, defined as the passage of > 3 watery stools in a 24-hour period, for ≥ 14 days.

4.26 Failure to thrive, defined as a drop in ≥ two percentiles of weight over a period of 2-3 months, corrected for gestational age.

4.27 Presence of contraindications for use of INH or TMP/SMX.
4.28 Receipt of any disallowed medications listed in Section 4.4.

4.29 Known or suspected diseases of the immune system other than HIV infection

4.210 Current or previous diagnosis of, or treatment for, malignancy.

4.211 Current immunosuppressive therapy, defined as > 1 mg/kg/per day of prednisone or its equivalent.

4.212 Anticipated long-term oral or intravenous corticosteroid therapy (> 3 weeks). Those receiving nonsteroidal anti-inflammatory agents and inhaled corticosteroids are not excluded

4.213 Other acute or chronic conditions that in the opinion of the investigator, may interfere with the achievement of the protocol objectives.

4.214 ≥ Grade 2 AST/SGOT, ALT/SGPT, ANC, platelet count, rash, neuropathy or myopathy at pre-entry.

4.215 ≥ Grade 3 hemoglobin at pre-entry.

4.216 Any Grade 4 clinical or laboratory toxicity within 14 days prior to entry.

4.3 Allowed Medications

Receipt of antiretroviral therapy, multivitamins, oral antibiotics, antimalaria medications, and antiparasitic drugs, will be allowed. Treatment with medications (not listed in Section 4.4, Disallowed Medications) that are known not to interfere with INH therapy will also be allowed.

Any change in TB or PCP prophylaxis, any initiations or changes in a study participant’s antiretroviral regimen, or any changes in other drug regimens after enrollment should be sent to the protocol team by e-mail at actg.teamp1041@fstrf.org.

4.4 Disallowed Medications

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>South African Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Tegretol</td>
</tr>
</tbody>
</table>
Phenytoin  Epanutin  
Diazepam  Valium  
Antacids (Aluminum salts)  Malos  
Prednisolone\(^1\) >3 weeks of continuous use  Prelone  
Prednisone\(^1\) >3 weeks of continuous use  Meticorten and Pulmison  
Cycloserine  
Rifampin

\(^1\) Topical and inhaled steroids are allowed.

Refer to the package inserts for medications disallowed with dapsone and atovaquone.

4.5 Use of Antiretroviral Therapy

The administration of antiretroviral therapy (ART) is not a part of this clinical trial. The use antiretrovirals, if available, will be according to country specific guidelines and is not criteria for exclusion from participation in this study.

The potential impact of the use of ARVs on the incidence of study endpoints is from 1) the decrease in TB infection/disease incidence because of improved immune function, and 2) the decrease in transmission because of fewer infected infants or lower infectivity. As reported by Williams, the latter is expected to have little impact even among the HIV-infected infants because the proportion of transmission from dually infected individuals is below 10% (95). Improved immune functioning, however, may have an impact on the background rate of endpoints. The actual impact depends on what CD4 level triggers the initiation of antiretroviral therapy and the degree of coverage for infants in this study.

Using the model proposed by Williams and assuming that starting ARVs at CD4% <20% for infants < 18 months or <15% for infants > 18 months is similar to starting ARVs in adults with CD4 <200 cells/mm\(^3\) and assuming 100% coverage of all infants meeting eligibility criteria, the impact is expected to be a decrease in cumulative incidence of 22%. With this decrease in incidence for the HIV-infected stratum, a reduction due to INH prophylaxis of 83% is needed to detect it with 80% power in the sample size of 500 (allowing 15% loss to follow-up). Lower coverage or smaller decreases in cumulative incidence will permit detection of smaller effects.

Based on projections derived by Williams, it is unlikely that the rollout of ARVs will appreciably reduce the incidence of TB infection or disease. The
impact on TB incidence from dually infected individuals is low and the degree of medication compliance and coverage of ARVs would both have to be high to have an appreciable effect.

The conservative assumptions are based on models developed by Williams from data conducted in adult cohort studies. The authors did not provide sufficient information to determine the estimated models exactly. Extreme caution is warranted because extrapolating adult findings to children may be inappropriate, and therefore the rate of endpoints in this cohort should be monitored carefully.

4.6 Enrollment Procedures

Protocol registration must occur through the Regulatory Compliance Center Protocol Registration Office before study participants can be enrolled in this study. A site implementation plan (SIP) is required prior to site participation in the study. The SIP must describe (1) the annual number of HIV-infected pregnant women who receive prenatal care and/or deliver at the site; (2) the anticipated number of HIV-infected children born to these women; (3) the anticipated number of study participants to be recruited at each site; (4) the anticipated percentage of women who refuse participation for their infants, and (5) a proposed plan for the retention of study participants. The SIP will be reviewed and approved by members of the protocol team and sites will be notified that authorization to participate has been granted.

At Johannesburg, there are approximately 30,000 births annually at the Chris Hani Baragwanath Hospital and related antenatal clinics. Of these, approximately 90% of the mothers consent to HIV testing in structured voluntary counseling and testing (VCT) programs administered by the Perinatal HIV Research Unit. The prevalence of HIV infection among women attending antenatal care is about 31%. Based on a transmission rate of 13%, it is anticipated that approximately 8,400 HIV-infected women will be identified annually and that 1,092 infants born to these women will be infected. Mothers will be informed of the study at the time they are told of their infection status and during subsequent antenatal visits. Mothers will be offered participation of their infants at birth and will be asked to return to the well-baby clinic run by the Perinatal HIV Research Unit where HIV-exposed children are offered routine childhood vaccinations. Informed consent for participation into the study will be obtained during well-baby clinic visits. Following agreement for participation into the study, infants will be screened for eligibility. Children who are referred from other services who meet inclusion criteria will also be eligible for participation.
At Cape Town, approximately 17,000 deliveries occur per year with seroprevalence varying from 7 to 15%. Approximately 1,470 HIV-exposed infants are born each year and, based on an estimated transmission rate of 13%, about 190 infants will be infected. Approximately 95% of the women consent to voluntary counseling and testing, and approximately 1% of the women elect to exclusively breastfeed. Expectant mothers at antenatal clinics will be informed about the study, reinforcing the message at delivery and again when the infant is two weeks at age. Neonates are seen at two weeks of age at community-based clinics and again at 6 weeks of age for their first immunizations. Mothers will be given appointments to attend a study enrollment visit at Children’s Infectious Diseases Clinical Research Unit (KID-CRU) at Tygerberg Children’s Hospital. Immunizations will be offered at this visit in order to minimize healthcare visits and TMP/SMX prophylaxis will be initiated.

At Durban, there are approximately 9000 births annually at the King Edward VIII Hospital. Of these, approximately 90% of the mothers consent to HIV testing in structured voluntary counseling and testing (VCT) programs administered by antenatal clinic staff as part of the national PMTCT programme. The prevalence of HIV infection among women attending antenatal care is about 40%. Based on a transmission rate of 10%, it is anticipated that approximately 3600 HIV-infected women will be identified annually and that 360 infants born to these women will be infected. Mothers will be informed of the study at the time they are told of their infection status and during subsequent antenatal visits. Mothers will be offered participation of their infants at birth and will be asked to return to the follow-up clinic run by Paediatric HIV team, where HIV-exposed children are offered routine care. Informed consent for participation into the study will be obtained during clinic visits. Following agreement for participation into the study, infants will be screened for eligibility. Children who are referred from other services (Paediatric Outpatient Clinic, Paediatric general wards), who meet inclusion criteria, will also be eligible for participation.

At Malawi, approximately 23,000 women attend antenatal clinics in the catchment area served by the UNC project. One hundred percent of the women accept HIV testing as part of the PMTCT program. The prevalence of HIV in this setting is 15%. From the 3500 projected HIV-infected mothers identified through the program, a transmission rate of approximately 15% and 525 HIV-infected infants will be identified at this site. Infants are tested at 6 weeks of
age through the antenatal and under-5 clinics and those testing HIV positive will be referred to this study.

At Gaborone, Botswana, there are approximately 10,000 deliveries a year in the Kgatleng district, South East district, Lobatse Township, Kanye and Mogoditshane. The HIV seroprevalence rate among pregnant women is about 35%, implying that approximately 3,500 HIV-exposed newborns are delivered per year. About 20% of all HIV-infected women are on HAART during pregnancy resulting in reduction of PMTCT to as low as 1-2%. The remaining 80% (2,800) are given the government sponsored zidovudine mono-prophylaxis regimen and HIV transmission rates are about 6%. Therefore, the approximate number of HIV-infected infants seen in the Gaborone catchment area is 170 per year. At Molepolole, there are approximately 4,000 deliveries per year within the catchment areas of the Kweneng district and at least 70 HIV-positive infants per year are identified.

Annual accrual at sites will be competitive, but it is anticipated that between 66-75% of HIV-exposed infected and uninfected children will be recruited at Johannesburg with the remainder being recruited at other international sites from high tuberculosis endemic regions being solicited for participation. Only study participants recruited at Cape Town and Durban will participate in the pharmacokinetics study and only study participants recruited at Johannesburg will participate in the pneumococcal colonization study. The number of study participants being recruited at each site will be reviewed every three months by the P1041 team, and will be subject to adjustment based on the site’s ability to accrue more or less than the number of projected subjects. Continued assessment of the number of HIV-infected children enrolled will be conducted to insure that the necessary 168 HIV-infected children are available for the population PK study. The HIV-exposed uninfected cohort closed to accrual on June 7, 2006 under Version 1.0 of the protocol and no more HIV-exposed uninfected infants will be enrolled in this study.

4.61 Enrollment Log

Each site must maintain an enrollment log of all infants born to HIV-infected mothers who are being followed at a clinic affiliated with a site and who have been informed of the study (See Appendix XII). The enrollment log will collect limited summary data on mothers and infants at time of first presentation to the well-baby clinic, such as age of the mother, and history of antiretroviral
use before and during pregnancy, race/ethnicity of the child, and reasons for refusal to participate (such as time and distance, concerns about confidentiality, mistrust in research, participation in a conflicting protocol, or specimen collection, noneligibility, nonavailability of guardian, and noncompliance with study procedures or visits). Each site will be required to submit grouped summary data for each calendar quarter to the IMPAACT Data Management Center. Mothers who refuse participation of their infant will be asked to allow the collection of demographic and medical information that will not identify the infant by name.

4.7 Co-enrollment

Co-enrollment in primary therapy protocols will be permitted, if all protocol chairs involved are informed of and agree to co-enrollment.

5.0 STUDY TREATMENT

5.1 Drug Regimens, Administration, and Duration

NOTE: There must be at least a 90-day window between receipt of BCG and the initiation of INH or INH placebo. Study participants are to take medication according to the arm to which they have been randomized to (INH vs. INH placebo). The study participant’s age and infection status will determine whether or not he/she will receive TMP/SMX.

Antiretroviral agents (and their administration) are not a part of this clinical trial, and will therefore NOT be provided by this study.

5.11 Drug Regimen

5.111 HIV-Infected Study Participants (INH)

- Isoniazid (INH): 10-20 mg/kg orally, once a day, using the dosing chart in Table 1. INH will be started any time beginning between the 91st –120th day of life.

- Trimethoprim/Sulfamethoxazole (TMP/SMX): 5 mg/kg of the trimethoprim component orally, once a day, until one year of age.
• Trimethoprim/Sulfamethoxazole (TMP/SMX): 5 mg/kg of the trimethoprim component orally, once a day, will continue after one year of age for HIV-infected children who meet WHO guidelines for continuing TMP/SMX prophylaxis

5.112 HIV-Infected Study Participants (Placebo)

• Placebo for INH: Administer orally, once a day, using the dosing chart in Table 1. Placebo will be started any time beginning between the 91st –120th day of life.

• Trimethoprim/sulfamethoxazole (TMP-SMX): 5 mg/kg of the trimethoprim component orally, once a day, until one year of age.

• Trimethoprim/sulfamethoxazole (TMP-SMX): 5 mg/kg of the trimethoprim component orally, once a day, will continue after one year of age for HIV-infected children who meet current WHO guidelines for continuing TMP/SMX prophylaxis

5.113 HIV-Exposed, Uninfected Study Participants (Isoniazid)

• Isoniazid (INH): 10-20 mg/kg orally, once a day, using the dosing chart in Table 1. INH will be started any time beginning between the 91st –120th day of life.

• Trimethoprim/Sulfamethoxazole (TMP/SMX) 5 mg/kg of the trimethoprim component orally, once a day until HIV status is confirmed by a repeat HIV DNA PCR and the child is no longer at risk for acquiring transmission from breast-feeding.

5.114 HIV-Exposed, Uninfected Study Participants (Placebo)

• Placebo for Isoniazid: Administer orally, once a day, using the dosing chart in Table 1. Placebo for INH will be started any time beginning between the 91st –120th day of life.
• Trimethoprim/Sulfamethoxazole (TMP/SMX) 5 mg/kg of the trimethoprim component orally, once a day until HIV status is confirmed by a repeat HIV DNA PCR and the child is no longer at risk for acquiring transmission from breast-feeding.

Table 1: INH and Placebo Dosing Chart

<table>
<thead>
<tr>
<th>Weight Ranges (kg)</th>
<th>Prescribed Isoniazid Dose (mg)</th>
<th>Number of 100 mg Tablets of INH or Placebo to be Administered per dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7-5</td>
<td>50</td>
<td>½ tablet</td>
</tr>
<tr>
<td>5.1-9</td>
<td>100</td>
<td>1 tablet</td>
</tr>
<tr>
<td>9.1-13</td>
<td>150</td>
<td>1 ½ tablet</td>
</tr>
<tr>
<td>13.1-18</td>
<td>200</td>
<td>2 tablets</td>
</tr>
<tr>
<td>18.1-23</td>
<td>250</td>
<td>2 ½ tablets</td>
</tr>
<tr>
<td>&gt;23</td>
<td>300</td>
<td>3 tablets</td>
</tr>
</tbody>
</table>

5.12 Drug Administration

• Study participants will be weighed at each study visit and prescribed a dose according to the dosing chart in Table 1.

• Instructions on how to crush INH tablets for administration are described in Appendix XI, Instructions for Crushing INH Tablets for Administration.

• INH/placebo will be administered mixed in water and the study participant should drink additional clear water to ensure that all of medication is in the stomach.

• INH/placebo should be given 30 minutes before a feeding, or 2 hours after a feeding.

• Study participants who vomit or spit up the medication in less than 45 minutes will be given a second dose at that time. Those who vomit or spit up after 45 minutes are considered to have absorbed the medication.

• No other liquid (including breast milk, juice, or medicine suspension) should be administered within one hour before or two hours after the administration of INH for the PK study. If this cannot be avoided, the amount and time of any liquid other than water must be recorded on the case report form.
• Antacids or buffered DDI should be administered at least three hours before or after the administration of INH.

5.121 Isoniazid/Isoniazid Placebo Administration

The correct number of isoniazid/isoniazid placebo tablets or half tablets will be broken into pieces no larger than ¼ tablet. The pieces will be crushed and then mixed with warm or cool water for administration. Since isoniazid/placebo mixes better in warm water than cool water, it is better to use warm water to prepare the isoniazid/placebo mixture for administration whenever possible. The resulting mixture will be drawn into an oral syringe and administered into the side of the mouth of the study participant. The resulting mixture must be administered within 30 minutes of mixing and will be administered once a day.

5.122 Trimethoprim/Sulfamethoxazole (Cotrimoxazole, TMP/SMX)

TMP/SMX: 5 mg/kg or the age equivalent weight band of the trimethoprim component of the suspension (or tablet if the study participant is of correct weight and can swallow tablets) will be administered into the side of the mouth of the study participant. TMP/SMX will be administered once a day.

5.2 Drug Formulation

5.21 Isoniazid/ Isoniazid Placebo

100 mg tablets and matching placebo will be manufactured by Be-Tabs Pharmaceuticals (Pty) Ltd. of the Republic of South Africa. INH and placebo tablets will be scored once to allow standard dosing of 50, 100, 150, 200, 250 and 300 mg increments. Tablets should be stored in a cool dry place below 25°C, protected from sunlight, and kept out of reach of children.

5.22 Trimethoprim/Sulfamethoxazole (Cotrimoxazole, TMP/SMX)

8 mg trimethoprim and 40 mg sulfamethoxazole per 1 ml suspension will be the most common formulation of TMP/SMX administered. For study participants
in whom the use of 80 mg trimethoprim and 400 mg sulfamethoxazole tablets is more appropriate, TMP/SMX tablets may be given.

5.3 Drug Supply, Distribution, and Pharmacy

5.31 Procurement of Isoniazid/Placebo

Isoniazid (100 mg tablets) and matching isoniazid placebo will be manufactured by Be-Tabs Pharmaceuticals (PTY) LTD, Roodepoort, South Africa. The isoniazid and matching isoniazid placebo will be purchased by Mark Cotton, Cape Town, South Africa for use in this protocol.

5.32 Distribution of Isoniazid/Placebo

Isoniazid tablets 100 mg and matching isoniazid placebo will be available through the site pharmacist.

Site pharmacists should contact the following people for additional supply of study drug:

Marlize Smuts, Pharmacist, at 021 938 4298, or
Joan Coetzee at 021 938 4157, or
Mark Cotton at 021 938 9127

Johannesburg
Pharmacists will dispense isoniazid/placebo tablets to field workers on a monthly basis. The field worker then will disseminate the tablets to the parent/guardian of the study participants. For those parents choosing to collect their own medications, the pharmacist will dispense a monthly supply of isoniazid/placebo to the parent/guardian.

Cape Town
Pharmacists will directly dispense a 3-month supply of isoniazid/placebo tablets to the parent/guardian of the study participant.

Durban
Pharmacists will directly dispense a 1-month supply of isoniazid/placebo tablets to the parent/guardian of the study participant. Pharmacists will directly dispense a 1-month supply of isoniazid/placebo tablets to the
A research assistant who will give the tablets to the parent/guardian of the study participant at the study clinic. Pharmacists will directly dispense isoniazid/placebo tablets to field workers on a monthly basis who will then disseminate the tablets to the parent/guardian of the study participant. A repeat prescription will be issued to cover 3 months supply of medication so the first issue will be directly to the parent/guardian of participant. The second and third issues will go either to parent/guardian, research assistant or field worker depending on the circumstances regarding problems such as transport. The study coordinator will keep the pharmacy informed of scheduled appointments and method of delivery regarding second and third month repeats. The prescription will supply all the details of the patient, drug and dose.

Malawi
Pharmacists will directly dispense a 3-month supply of isoniazid/placebo tablets to the parent/guardian of the study participant.

5.33 Trimethoprim/Sulfamethoxazole (Cotrimoxazole, TMP/SMX)

Trimethoprim/Sulfamethoxazole will be supplied through the P1041 study. Each site will be responsible for obtaining the necessary TMP/SMX supplies for their study participants necessary for administration.

5.34 Dapsone and Atovaquone

Dapsone and atovaquone will NOT be supplied by the study.

5.35 Pharmacy and Study Product Accountability

The CTU pharmacist is required to maintain complete records of all study medication received for the P1041 protocol. The unused commercially available INH tablets that retain good expiration dating will be donated to the local site for use for prophylaxis treatment after the trial. The CTU pharmacist will arrange for the destruction of the unused placebo tablets. The procedures to follow for accountability can be found in the manual, Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

5.4 Study Product Labeling
Isoniazid and placebo must be labeled in a blinded fashion. Suggestions for blinded labeling are:

- P1041 Study Medication; or
- Isoniazid/Placebo Study Medication.

### 6.0 SUBJECT MANAGEMENT

#### 6.1 General Toxicity Management

The Division of AIDS (DAIDS) Table for Grading the Severity of Pediatric Adverse Events (Appendices IVA-B) will be used to screen for eligibility and to grade clinical and laboratory toxicities and can be found at [http://rcc.tech-res-intl.com](http://rcc.tech-res-intl.com). The Supplemental Toxicity Table for Grading the Severity of Peripheral Neuropathy (Appendix V) will be used to grade symptoms of peripheral neuropathy (see Section 6.4). For all Grade 3 and 4 clinical and laboratory adverse events, notify the protocol team at actg.teamp1041@fstrf.org of the initial adverse event and the repeat clinical observation or laboratory values.

Management of INH and TMP/SMX (study drugs) dosing will take into consideration whether the toxicity is believed to be study drug(s) related. The study participant should be followed until the toxicity resolves to ≤ Grade 2.

Grade 1 - Continue study drugs; routine monitoring.

Grade 2 - Continue study drugs; monitor closely with more frequent visits; work-up to exclude other causes.

Grade 3 - For all Grade 3 abnormalities, **(except hemoglobin)**, every effort should be made to have the study participant observed clinically and/or have repeat laboratory tests performed within 72 hours of the event, or as soon as possible. **For Grade 3 hemoglobin in subjects for whom there has not been more than a 1.0gm decline between pre-entry and current value, a repeat test should be done within two weeks. If repeat hemoglobin is still <1.0gm decrease from baseline value, resume normal screening.**

For Grade 3 laboratory abnormalities, study participants should continue taking study drugs pending receipt of repeat laboratory tests. However, the clinician has the option to immediately interrupt study drugs if a repeat laboratory test cannot be performed within 72 hours, or if the clinician determines that
continuation of study drugs is unsafe while awaiting clinic or test results. For Grade 3 toxicities supported by repeat clinical observation or laboratory testing, hold study drug and contact the protocol team at actg.teamp1041@fstrf.org to determine course of action. For course of action for Grade 3 hematologic, hepatic, dermatologic and peripheral nervous system abnormalities, see sections 6.21, 6.22, 6.3, and 6.4 respectively.

Grade 4 - For Grade 4 hematologic, hepatic, dermatologic, and peripheral nervous system abnormalities hold study drugs immediately and, see sections 6.21, 6.22, 6.3, and 6.4 respectively. Every effort should be made to have the study participant observed clinically and/or obtain repeat laboratory results within 72 hours of the event. If this is not possible, re-evaluation should be done as soon as possible and the protocol team notified of the results.

For all other Grade 4 abnormalities, hold study drugs immediately and contact the protocol team at actg.teamp1041@fstrf.org to determine course of action. Every effort should be made to have the study participant observed clinically and/or obtain repeat laboratory results within 72 hours of the event. If this is not possible, re-evaluation should be done as soon as possible and the protocol team notified of the results.

6.2 Protocol Specific Toxicity Management

The toxicity management plans follows the local standard of care at the South African sites (i.e., substitution of alternative PCP prophylaxis in case of laboratory toxicity associated with TMP/SMX). Also, it should be noted that Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is not present in the South African population. It is assumed that with each medication change as per the above algorithms, ≤ 5% of study participants beginning a new medication would have a ≥ Grade 3 toxicity. This assumption would be assessed monthly by the study team.

6.21 Hematologic Abnormalities

A complete blood count (CBC) will be obtained every three months in study participants who are receiving TMP/SMX or other PCP prophylaxis. If or when PCP prophylaxis is discontinued, CBC testing will be discontinued. CBCs will NOT be monitored in any study participants who are NOT receiving PCP prophylaxis.
For ≥ Grade 3 hematologic abnormality in study participants receiving TMP/SMX, the following algorithm should be followed. INH/Placebo will NOT be discontinued for management of hematologic abnormalities.

- TMP/SMX will be held for two weeks and a CBC will be obtained.

If ≥ Grade 3 abnormality is still present after two weeks of the initial abnormal value, discontinue TMP/SMX and substitute with dapsone 2mg/kg once a day.

If the initial ≥Grade 3 abnormality has resolved to ≤ Grade 2 within the two weeks after the date that TMP/SMX has been held, restart TMP/SMX. Obtain a CBC two weeks later to determine whether a toxicity ≥ Grade 3 has occurred. If it has, discontinue TMP/SMX and initiate dapsone prophylaxis.

- If dapsone was used to replace TMP/SMX, obtain a CBC two weeks after starting dapsone. If toxicity has not resolved to ≤ Grade 2, discontinue dapsone and initiate atovaquone 40 mg/kg three times a day with meals.

- If atovaquone has been used to replace dapsone, obtain a CBC two weeks after starting atovaquone. If toxicity has not resolved to ≤ Grade 2, the best alternative, available strategy for prophylaxis of PCP will be offered by the investigator. The CBC should be followed until resolution of the toxicity to ≤ Grade 2.

6.22 Hepatic Abnormalities

In the case of significant hepatic dysfunction, study participants will be screened for infection due to hepatitis A or B, as is the current clinical practice. Study participants also may be screened for other potential causes of hepatic dysfunction, as per clinical indication.

For the purposes of the current study, study participants who present with symptoms suggestive of hepatic dysfunction will be screened for possible herbal medications, by way of interview with the mother. Study participants will be
screened for the use of herbal medications because herbal medications have been associated with liver toxicity. In most instances, however, a history suggestive of such an association is available.

6.221 Liver Function Tests (LFTs) in study participants on PCP prophylaxis - Serum Glutamic Oxalacetic Transaminase (SGOT) Aspartate Amino Transferase (AST) and Serum Glutamate Pyruvate Transaminase [(SGPT) Amino Alanine Transferase (ALT)]: (AST = SGOT; ALT = SGPT)

Elevated serum transaminases (SGOT, SGPT), hyperbilirubinemia, bilirubinuria, jaundice, and occasionally severe and sometimes fatal hepatitis have been reported with INH (54). INH-associated hepatotoxicity is rare in children. If it occurs, INH-associated hepatotoxicity usually occurs in the first two to three months after starting the drug.

Study participants will have LFTs measured every three months while receiving TMP/SMX or any other PCP prophylaxis. LFTs will be obtained every six months if/when PCP prophylaxis is discontinued.

Note: If there is an increase of LFTs to > Grade 3, follow the study participant until resolution of the toxicity to ≤ Grade 2.

For ≥ Grade 3 hepatic abnormality supported by repeat laboratory tests:

- Hold TMP/SMX and INH/placebo for two weeks and recheck LFTs in 2 weeks.

- If a ≥ Grade 3 abnormality is still present after withholding TMP/SMX and INH/placebo for two weeks:
  - Discontinue TMP/SMX and initiate dapsone. (Hold INH/placebo.)
• Obtain LFTs two weeks after the initiation of dapsone. If a ≥ Grade 3 abnormality persists, discontinue dapsone and initiate atovaquone. (Hold INH/placebo.)

• Obtain LFTs two weeks after the initiation of atovaquone. If a ≥ Grade 3 abnormality persists, permanently discontinue atovaquone and INH/placebo but remain on study.

• If toxicity has resolved to ≤ Grade 2 after introduction of dapsone or atovaquone, INH/placebo will be restarted and LFTs will be rechecked in 2 weeks. If toxicity is ≤ Grade 2 two weeks after INH/placebo is restarted, INH/placebo and PCP prophylaxis will continue. If a toxicity is ≥ Grade 3 two weeks after INH/placebo is restarted, INH/placebo will be discontinued but the study participant will remain on study. LFTs should be followed until resolution of the toxicity to ≤ Grade 2.

• If the toxicity has resolved to ≤ Grade 2 after TMP/SMX and INH/Placebo have been held for two weeks:
  
  • Restart TMP/SMX and obtain LFTs two weeks after the initiation of TMP/SMX.

• If toxicity remains ≤ Grade 2 for two weeks after the reintroduction of TMP-SMX:
  
  • Restart INH/Placebo and obtain LFTs two weeks later.

  • Continue INH/placebo and TMP/SMX if toxicity remains ≤ Grade 2 two weeks after the reintroduction of INH/placebo.

  • Discontinue INH/placebo, if after two weeks on INH/Placebo, toxicity is ≥ Grade 3. Study participant will remain on-study. LFTs should be followed until resolution of the toxicity to ≤ Grade 2.
• If there is a ≥ Grade 3 toxicity two weeks after re-introducing TMP-SMX, stop TMP-SMX and start dapsone and follow algorithm above.

**NOTE:**
In the event of ≥ Grade 3 hepatic toxicity, investigators may consider evaluating for other causes of elevated LFTs (e.g., hepatitis A virus infection).

6.222 LFTs in HIV-infected study participants not on PCP prophylaxis and HIV-uninfected study participants not on PCP prophylaxis

HIV-infected study participants not on PCP prophylaxis and HIV-uninfected study participants not on PCP prophylaxis will have LFTs measured every six months while receiving INH/placebo.

In the event of ≥ Grade 3 hepatic toxicity, LFTs should be followed until resolution of the toxicity to ≤ Grade 2.

In the event of ≥ Grade 3 hepatic toxicity, investigators may consider evaluating for other causes of elevated LFTs (e.g., hepatitis A virus infection).

• For ≥ Grade 3 hepatic toxicity, discontinue INH/placebo and obtain LFTs two weeks after suspension of INH/placebo.

  • If the toxicity has not resolved to ≤ Grade 2:
    • Hold INH/placebo for an additional two weeks and obtain LFTs at the end of the two weeks.
  • If the toxicity has still not resolved to ≤ Grade 2:
    • Discontinue INH/placebo. Continue study participant on study.
  • If the toxicity resolves to ≤ Grade 2 after holding INH/placebo for two to four weeks:
• Reintroduce INH/placebo and obtain LFTs two weeks after reintroduction.

• If toxicity remains \( \leq \) Grade 2 two weeks after reintroduction of INH/placebo
  
  • Continue INH/placebo.

• If two weeks after the reintroduction of INH/placebo, toxicity is \( \geq \) Grade 3:
  
  • Discontinue INH/placebo. Study participants will remain on study.

6.3 Skin Rash

6.3.1 Study participants on PCP prophylaxis

Note: In the event of \( \geq \) Grade 3 rash, the rash should be followed until resolution to \( \leq \) Grade 2.

• For \( \geq \) Grade 3 rash:
  
  • Hold TMP/SMX and INH/placebo until resolution of the rash.

  • After the rash resolves:
    
    • Discontinue TMP/SMX
    • Initiate dapsone prophylaxis

  • If a \( \geq \) Grade 3 rash recurs:
    
    • Discontinue dapsone.

  • When the rash resolves after the discontinuation of dapsone:
    
    • Initiate atovaquone prophylaxis

  • If \( \geq \) Grade 3 rash recurs:
• Discontinue atovaquone and INH/placebo. Study participant will remain on study.

• If the rash does not recur following two weeks after the introduction of dapsone or atovaquone:
  • Restart INH/placebo.

• If after restarting INH/placebo, ≥ Grade 3 rash recurs:
  • Discontinue INH/placebo. Study participant will remain on study.

6.32 HIV-infected study participants not on PCP prophylaxis and HIV-uninfected study participants not on PCP prophylaxis

Note: In the event of ≥ Grade 3 rash, the rash should be followed until resolution of the toxicity to ≤ Grade 2.

• For ≥ Grade 3 rash:
  • Hold INH/placebo until the resolution of rash.

• When the rash resolves:
  • Restart INH/placebo.

• If a ≥ Grade 3 rash recurs after restarting INH/placebo:
  • Discontinue INH/placebo. Study participant will remain on study; however, the physician should weigh the risk of discontinuing all PCP prophylaxis for HIV-infected infants and consider whether remaining in the study is in the infant’s best interest.

6.4 Peripheral Neuropathy

6.41 Pyridoxine Supplementation and INH
The 2003 American Academy of Pediatrics (AAP) Redbook recommends the use of pyridoxine (Vitamin B6) for children and adolescents treated with INH who are on meat and milk-deficient diets and for those with nutritional deficiencies, including all symptomatic HIV category C disease children, infants who are exclusively breastfed, and their mothers. The U.S. current standard of practice does not recommend giving pyridoxine (Vitamin B6) to infants who are on INH prophylaxis.

NOTE:
The truncated Denver Developmental Test (Appendix VI, “Evaluation of Peripheral Neuropathy”) and the Supplemental Toxicity Table for Grading Severity of Peripheral Neuropathy (Appendix V) will be used for determining the presence and grading of peripheral neuropathy.

For Grade 2 peripheral neuropathy:

- Continue INH/placebo
- Continue dapsone (if being given for PCP prophylaxis)
- Reassess within 4 weeks

For Grade 3 peripheral neuropathy:

- Continue INH/placebo
- Continue dapsone (if being given for PCP prophylaxis)
- Start pyridoxine (Vitamin B6) 25-50mg per day
- Reassess within 2 weeks.

- If ≥ Grade 3 symptoms persist for > 2 weeks:
  - Discontinue INH/placebo
  - Discontinue dapsone
  - Continue pyridoxine (Vitamin B6)

- Once symptoms resolve to ≤ Grade 2:
  - Restart INH/placebo
  - Restart dapsone
  - Continue pyridoxine (Vitamin B6)
  - If ≥ grade 3 symptoms recur, stop INH/placebo, continue pyridoxine, continue on study.

For Grade 4 peripheral neuropathy:
- Hold INH/placebo
- Hold dapsone
- Start pyridoxine (Vitamin B6)
- Reassess within 2 weeks. Consider nerve conduction tests.

- Once symptoms resolve to ≤ Grade 2:
  - Restart INH/placebo
  - Restart dapsone
  - Continue pyridoxine (Vitamin B6)
  - If ≥ grade 3 symptoms recur, discontinue INH/placebo, continue pyridoxine, continue on study.

**NOTE:**
In the event of a > Grade 3 peripheral nervous system toxicity, investigators should evaluate for other causes or insure that appropriate referral is made.

6.5 **TB Exposure and TB Disease Management**

All study participants found to be TB infected will discontinue study treatment (but not be unblinded), remain on study, and be given appropriate treatment according to the country specific Tuberculosis Control Program guidelines. Department of Health clinics are located in all areas where this trial is being conducted and there is no barrier to care at these clinics. INH is currently approved, available and administered free of charge in South Africa and Malawi for use as short-term prophylaxis (6-9 months) for children exposed to TB. If an evaluation is performed for suspected TB disease or infection, at either a scheduled study visit or an additional clinic visit, results of the evaluation are to be recorded on the appropriate study CRF for event tracking and entered in the database within 48 hours of the evaluation visit.

6.5.1 **Tuberculin Skin Testing (TST) – Purified protein derivative (PPD) preparation RT23-2TU --Mantoux Method**

* M. tuberculosis* delayed hypersensitivity testing (DTH) to detect reactivity will be performed according to accepted standards by the intradermal (ID) injection of 0.1 ml (100 µl) of RT23 2TU into the volar aspect of the left forearm. All TSTs must be read between 48 and 72 hours after placement. Readings of the horizontal diameter of the induration will be recorded in millimeters (mm) and the reading will be interpreted as being positive or negative based upon
previously established criteria specified in Appendix II (Algorithm to Diagnose Clinical Tuberculosis).

6.52 TB Exposure

6.521 Any study participant identified as exposed to a non-household contact with AFB smear positive and/or culture positive PTB will be evaluated for TB infection and disease. Study participants (all under five years of age) will be screened for disease using simple clinical criteria (e.g., cough, nutritional status, unexplained fever, including the performance of CXRs or TST). Those study participants found to have TB infection or disease will discontinue study drug but continue on study and will be referred for treatment according to country specific Tuberculosis Control Program guidelines.

If a study participant, post exposure to TB, has no clinical evidence of TB disease and has a negative TST and chest x-ray, the study team will recommend that the study participant remain on INH/placebo on study with the provision that long-term follow-up is assured. Study participants without TB infection will remain on INH/placebo and on study. If, the health care provider feels that the study participant should receive prophylaxis with INH by prescription, the study participant will remain on study, but off INH/placebo.

Any study participant identified as exposed to a non-household contact with sputum smear positive PTB should receive INH if daily contact with the source case was for at least two weeks prior to therapy, or if the source case was on therapy for less than one month. The study participant will discontinue INH/placebo, but remain on study and should be retested in three months.

6.522 Any study participant identified as exposed to a non-household AFB sputum smear-negative and culture-negative case of PTB will be evaluated for evidence of TB disease:

- In the presence of TB infection or disease, the study participant will stop INH/placebo and be referred to the local TB control program
for evaluation and treatment as indicated per standard of care, but remain on study.

- In the absence of TB infection and disease, the study participant will continue on INH/placebo and on study.

6.523 Any study participant identified as exposed to a house-hold case of active TB whether household contact is smear or culture positive or negative, will be referred for prophylaxis and will be discontinued from INH/placebo but remain on study. Documentation of TB in the index household will be obtained. Every effort should be made to ensure that mothers with active TB are identified before the start of the study.

6.53 TB Disease

The Algorithm to Diagnose Clinical Tuberculosis (Appendix II) is to be used to screen for pulmonary TB at each study visit. Study participants who score greater than or equal to 4 on the algorithm during routine screening (excluding TST) will be screened further with TST and a chest x-ray (CXR).

If a study participant develops TB disease, the protocol team will notify the attending physician that the study participant may have been receiving INH during the course of the study. The protocol team will recommend that the study participant be treated with a combination of INH, rifampicin (RIF) and pyrazinamide (PZA), and, ethionamide.

6.6 Clinical Pneumonia

Study participants who have evidence of rales on chest wall auscultation; or tachypnea (>50 breaths per minute in infants, and >40 breaths per minute in other children with lower chest wall indrawing); or any airspace consolidation on chest radiograph will be categorized as having pneumonia.

All study participants who present with symptoms should have the following work-up:

- chest x-ray (CXR);
- TST–RT23 2 TU;
- transmembrane O₂ saturation in room air;
- routine blood culture for bacteria;
- complete blood count (CBC)
• induced sputum and nasopharyngeal aspirate for *P. jiroveci*
• induced sputum and gastric washings for *M.tb.* culture

NOTE:
Nasopharyngeal aspirates for respiratory viruses, using immunofluorescence and PCR techniques, will be done only at Johannesburg.

6.61 *Pneumocystis jiroveci* pneumonia (PCP)

Study participants with documented PCP based upon the presence of *P. jiroveci* cysts or trophozoites obtained from a broncho-alveolar wash, a deep endotracheal aspirate, and/or induced sputum, and/or nasopharyngeal aspirate will be treated with parenteral TMP/SMX as per established guidelines in South Africa. The protocol team should be notified of any cases of PCP.

Study participants diagnosed with PCP and/or bacterial pneumonia will continue to receive primary prophylaxis with INH/placebo and will remain on study.

6.7 Perinatal HIV Infection

Study participants with HIV infection will be followed according to country specific guidelines to monitor HIV disease status. Between scheduled visits, guardians are instructed to bring their infants to the HIV clinic whenever they become ill. Study participants with advanced disease or acute problems are seen more frequently. Study investigators provide or directly supervise acute and long-term care to ensure that disease progression is identified in a timely manner. Guardians of study participants will be instructed to seek care for symptoms that appear to be related to TB or HIV.

Study participants documented to be HIV-infected will be referred to the follow-up research clinic for long-term monitoring and clinical care based on the existing standard of care treatment guidelines for HIV. All HIV-1 infected study participants who develop progressive HIV disease or who develop an AIDS-defining illness will be reported to the protocol team. HIV-infected study participants less than 12 months of age will be started on TMP/SMX for PCP prophylaxis until 12 months of age. After 12 months of age, TMP/SMX will be continued according to country specific guidelines.

All study participants with a negative DNA PCR at pre-entry should receive
TMP/SMX (5mg/kg/dose of the trimethoprim component, orally once a day) until a follow-up DNA PCR is done and negative at 6 months of age. Based on the potential risk of post-natal HIV transmission from the mother to child, particularly if there is ongoing exposure to breast milk, all HIV-1 exposed uninfected children will also be continued on TMP/SMX prophylaxis at least until a repeat HIV DNA PCR test is performed at 6 months (24 weeks) of age in these children. Following confirmation of the child being HIV-uninfected at 6 months of age, TMP/SMX prophylaxis will be stopped in children that are not being breast-fed and continued among those still being exposed to breast-feeding. TMP/SMX prophylaxis will be continued in the latter group of children until HIV infection status has been determined based on EIA at the 60-week study visit. TMP/SMX prophylaxis will be continued among children in whom the HIV DNA PCR test is reactive at 6 months of age, and these children will subsequently be managed the same as other HIV infected children until infection status is resolved.

6.8 Criteria for Treatment Discontinuation

Study participants will be discontinued from study treatment but remain on study for any of the following reasons. The study participant will be treated per country specific standard of care, and PCP prophylaxis will be available/offered to those who meet WHO guidelines for PCP prophylaxis.

Study participants should complete study visits and laboratory evaluations scheduled according to Appendix IA and IB, except for pharmacokinetics and adherence measures.

6.81 the investigator determines that further participation would be detrimental to the study participant's health or well being.

6.82 the study participant requires treatment with medications that are disallowed while on study,

6.83 the study participant experiences drug toxicity necessitating study drug discontinuation as defined in Section 6.0

6.84 the study participant becomes infected with TB.

6.85 the study participant develops TB disease.
6.86 the study participant has no contact with study personnel for four consecutive months at either home medication visits or clinic medication visits.

6.87 the study participant requires INH prophylaxis due to household/ non-significant household smear positive/ culture positive exposure to TB.

6.88 the study participant requires treatment with oral or intravenous steroids for a continuous period > 3 weeks

6.9 Criteria for Treatment and Study Discontinuation

Study participants will be discontinued from study treatment and go off study for any of the following reasons.

Study participants should complete an off study form and all study laboratory evaluations as per week 96. This includes PPD testing (and return at 48-72 hours for reading of PPD), truncated Denver Developmental exam, adherence measures, liver function, hematology, virology, and stored sample. HIV RNA PCR and lymphocyte subsets should also be sent if patient is HIV-infected.

6.91 The parent/legal guardian refuses further treatment and/or follow-up evaluations

6.92 The HIV-infected study participant has no study visits in a 6-month period during the first 24 months of study

6.93 The study participant has no study visits within a 12-month period during the second 24 months of study

7.0 EXPEDITED ADVERSE EVENT REPORTING

The expedited adverse event (EAE) reporting requirements and definitions for this study and the methods for expedited reporting of adverse events (AEs) to the DAIDS Regulatory Compliance Center (RCC) Safety Office are defined in “The Manual for Expedited Reporting of Adverse Events to DAIDS” (DAIDS EAE Manual) dated May 6, 2004. The DAIDS EAE Manual is available on the RCC web site (http://rcc.tech-res-intl.com/aeae.htm).

AEs reported on an expedited basis must be documented on the DAIDS Expedited Adverse Event Reporting Form (EAE Reporting Form) available on the RCC web site. DAIDS EAE forms should be submitted to DAIDS through the Regulatory RCC
Safety Office (RCCSafetyOffice@tech-res.com) or call 301-897-1709 or fax 1-800-275-7619 or 301-897-1710. In addition, the site investigator is required to submit AE information as required by local regulatory or other local authority.

This study uses the Intensive Level of expedited AE reporting as defined in the DAIDS EAE Manual. The study agents that must be considered in determining the relationship of AEs requiring expedited reporting to DAIDS are INH, matched placebo, TMP/SMX, dapsone and atovaquone.

The Division of AIDS Table for Grading the Severity of Pediatric Adverse Events (< 3 months of age and > 3 months of age dated April 1994) must be used and is available on the RCC web site (http://rcc.tech-res-intl.com/eae.htm).

AEs must be reported on an expedited basis at the Intensive Level during the protocol-defined EAE Reporting Period, which is the entire study duration for an individual subject (from study enrollment until study completion or discontinuation of the subject from study participation for any reason).

After the end of the Protocol-defined EAE Reporting Period stated above, sites must report serious, unexpected, clinical suspected adverse drug reactions if the study site staff becomes aware of the event on a passive basis, i.e. from publicly available information.

8.0 STATISTICAL CONSIDERATIONS

8.1 Design

This is a multicenter randomized, double-blinded, placebo-controlled, Phase II/III clinical trial comparing INH versus placebo in children perinatally-exposed to HIV with stratification according to HIV infection status.

Five hundred HIV-infected study participants and 800 perinatally-exposed, HIV-uninfected study participants (closed to accrual as of June 7, 2006 under Version 1.0), approximately three months of age, will be randomized in a 1:1 ratio to INH and INH placebo.

The first 336 infants enrolled in the study at Cape Town and Durban who consent will participate in population PK and be randomized to one of four
sampling weeks and blood draw time combinations in a 1:1:1:1 ratio. Sampling will be at study weeks 0 and 84 or study weeks 12 and 84 and blood draw times at 1 and 3 hours post-dose or 2 and 4 hours post-dose. Half of the study participants enrolled in the population PK will be HIV-infected and half will be HIV-uninfected. To maintain blinding of clinical staff providing care to study participants, half of the study participants will be randomized to INH and half to INH-placebo. Laboratory staff will not be blinded to INH/INH placebo assignment so that only samples from study participants receiving active drug will be tested. No results will be transmitted back to clinicians providing care to study participants.

The randomization list will be created using permuted blocks, stratified by HIV infection status and whether or not the parent/guardian agreed to allow the study participant to undergo population PK. Randomization will be balanced by institution of enrollment.

Study participants will receive INH or INH placebo for 96 weeks and will then be followed for an additional 96 weeks.

8.11 Primary Endpoints

8.111 Time from randomization to development of TB disease or death censored at 96 weeks on study for HIV-infected children; and

8.112 Time from randomization to development of TB infection or death censored at 96 weeks on study for perinatally-exposed, HIV-uninfected children

8.12 Secondary Endpoints

Among both HIV-infected and perinatally-exposed, HIV-uninfected study participants:
8.121 Time from randomization to new first Grade 3 or worse sign or symptom, first Grade 3 or worse peripheral neuropathy, first Grade 3 or worse lab toxicity (i.e., hemoglobin, ANC, platelet, SGOT or SGPT), first Grade 3 or worse hemoglobin, first Grade 3 or worse ANC, first Grade 3 or worse platelet, Grade 3 or worse SGOT, Grade 3 or worse SGPT;

8.122 Time from randomization to development of TB disease censored at 96 weeks on study, and time from randomization to development of TB disease censored at 192 weeks;

8.123 Time from randomization to development of TB infection censored at 96 weeks on study, and time from randomization to development of TB infection censored at 192 weeks;

8.124 Time from randomization to development of TB disease or death censored at 192 weeks, and time from randomization to development of TB infection or death censored at 192 weeks;

8.125 Time from randomization to death censored at 96 weeks and time from randomization to death censored at 192 weeks;

8.126 Population PK model of INH as measured by molar concentrations of INH at two dosing interval time points on two separate occasions to determine absorption rate constant, apparent plasma clearance, and apparent volume of distribution.

Among HIV-infected study participants only:

8.127 Time from randomization to development of TB infection or death censored at 96 weeks;

8.128 Time from randomization to HIV disease progression or death censored at 96 weeks, and time from randomization to HIV disease progression or death censored at 192 weeks.

Among perinatally-exposed, HIV-uninfected study participants only:
8.129 Time from randomization to development of TB disease or death censored at 96 weeks on study.

8.13 Tertiary Endpoints

8.131 Time from randomization to diagnosis of PCP and time from randomization to PCP-related death for HIV-infected children;

8.132 Percentage of study participants with antibiotic susceptible *Streptococcus pneumoniae* isolated from nasopharyngeal swabs performed at 12, 24, 36, 72, 120, and 168 weeks on study. (Only at Johannesburg).

8.133 Resistance of *M. tb.* to INH among culture-confirmed TB disease cases at the time of diagnosis of disease.

8.2 Sample Size and Power Calculations

Although a loss to follow-up rate of approximately 4-5% is expected, the P1041 team conservatively assumes the loss-to-follow-up rate will be 15% over the two-year prophylaxis period for sample size and power calculations. We also assume that the loss-to-follow-up rate will be an additional 15% for the two-year post-randomized prophylaxis period. Comparisons across prophylaxis groups will be based on two-sided log rank tests with size 0.05. The log rank test will be used to compare prophylaxis arms because we are interested in comparing the time to some specific event (TB disease or death for the HIV-infected children and TB infection or death for the perinatally-exposed HIV-uninfected children) across these arms. The log rank test is sensitive to delays in the onset of specific events due to study drug since it takes into account when an event occurs rather than just whether or not it occurs. A test utilizing differences in proportion of study participants with the specific event at 96 weeks would have lower power or require that we obtain a larger sample size. The tables that follow are based on calculations that assume exponential distribution for the time to event distributions. For each of the selected sample sizes we present the power to detect difference in time to event between the control and the treatment arms given rates of the specific event during the first 96 weeks on study in the two arms, along with the expected number of events. The latter takes into account possible termination of study during interim monitoring.
Table 2 shows the power associated with a sample size of 500 to detect the effect of INH prophylaxis on time to TB disease or death for HIV-infected study participants assuming that the 96-week rate of TB disease or death in the control arm is 40% and the rate in the treatment arm varies from 25% to 35%. The event rate of 40% in the control arm comes from the assumptions of 10% TB disease rate and 30% rate of death without TB disease. In the INH arm a reduction of 75% in the 96-week TB disease rate is believed to be clinically significant, while a clinically significant reduction in the rate of death without TB disease is 25%. The effect of INH prophylaxis on death without TB disease could be a result of death related to TB infection without TB disease or death related to undiagnosed TB disease. Under this situation the 96-week event rate in the treatment arm is 25%, and the study has a power of 92% to detect the difference in time to event between the two arms. The power is also calculated under some smaller INH effects. When the 96-week rate of TB disease or death is 27.5% in the treatment arm the power is still satisfactory (79%).

In the power calculation above we have assumed the 96-week rate of death without TB in the control arm to be 30%. This is only a rough estimate, and we also present power under various settings with either a high death rate of 45% (Table 3) or a low death rate of 15% (Table 4) in the control arm. With the high death rate for the controls, the overall 96-week event rate is 55% (assuming a TB disease rate of 10% for the controls), and we have very high power (99%) to detect a clinically significant reduction of event rate for the INH recipients (75% reduction in the TB disease rate and 27.8% reduction in the rate of death without TB disease induces an overall event rate of 35%). For a smaller INH effect (40% event rate in the treatment arm) the power is still high (88%). With the low death rate of 15% for the controls, the overall 96-week event rate is 25% (assuming a TB disease rate of 10% in the control arm), and we have high power (92%) to detect a clinically significant reduction of event rate for the INH recipients (75% reduction in the TB disease rate and 33.3% reduction in the rate of death without TB disease induces an overall event rate of 12.5%). For a smaller INH effect (15% event rate in the treatment arm) the study still has moderate power (74%).

Although it is unlikely (the study team anticipates that at most 50 study participants will be affected), there is the possibility that antiretroviral drugs will become more widely available and therefore children on this study will start
antiretroviral therapy during the four-year follow-up period. Because of the interrelationship between TB and HIV, this would imply that larger sample sizes will be required to achieve the same power. However, the trial has been designed to assume a conservative rate of TB disease in the control arm and it is not anticipated that this will be a problem. If primary therapy decreases the magnitude of the effect of INH prophylaxis in addition to reducing the overall rate of disease, then an increase in sample size may be needed. With this in mind, the DSMB will be asked to review the rates of antiretroviral drug usage.

Tables 5 and 6 show the power associated with a sample size of 800 to detect a reduction of 75% or 50% in the 96-week rate of TB infection or death due to prophylaxis, using a broad range of failure rates in the control arm. It can be seen that the power to detect a 75% reduction in the failure rate is always satisfactory. When the failure rate is very low (5%) in the control arm, we have a good power of 82.5% to detect a 75% reduction in the treatment arm, and when the failure rate is 10% or higher in the control arm, the power to detect a 75% reduction is very high (>98%). Power to detect a reduction of 50% due to prophylaxis is lower, though we still have good power (>88%) to detect the reduction if the failure rate in the control arm is 15% or higher.

8.21 Factors Influencing Sample Size and Power

8.211 HIV-infected study participants

Data from South Africa’s Chris Hani Baragwanath Hospital suggest that the incidence of clinical disease due to *M.tuberculosis* among HIV-infected children, over the first two years of life, is 13.5% in the absence of INH prophylaxis. This calculation was derived from the incidence rates of culture-confirmed TB presenting as acute pneumonia in children less than 2 years of age, which is $1470/10^5(5)$. It was assumed that these cases of PTB that presented as acute pneumonia represented only one-third of cases of PTB in children, the remainder of whom would present with classical chronic symptoms. These figures result in a TB disease rate of approximately 13.5% over two years when considering that *M.tuberculosis* are isolated in less than one-third of children with PTB. However, because of the regular study visit schedule and the fact that children will be assessed more
frequently and thoroughly for TB, it is more likely that exposure to smear-positive cases will be identified among children enrolled on this study compared to those children in the target population. It is estimated that the rate of TB disease in the control arm will be at least 10% over two years. There is no definitive data about the two-year rate of death without TB disease for this specific population of HIV-infected children. The study team has decided that 30% would be a reasonable estimate, with 15% and 45% as the lower and upper bounds. In addition, the study also will be designed to determine the potential cost regarding the burden of TB among HIV-1 infected children, in addition to which an estimate would be derived regarding the cost of implementing an INH prophylaxis program. It is envisioned, that with the additional data, a cost-effectiveness analysis will be done in terms of determining at what level of efficacy it would be beneficial to introduce an INH prophylaxis program into the health-care program in South Africa.

8.212 Perinatally-exposed, HIV-uninfected study participants

Reliable estimates of rates of TB infection or death among HIV-exposed, but uninfected children are not available at this time. Power is calculated for a broad range of the 96-week failure rate. Decrease of the failure rate by 75% has the potential to have a significant public health impact that would make widespread use of INH prophylaxis cost effective. Therefore, we chose a conservative sample size of 800 (400 per treatment arm) in which to study prophylaxis in perinatally exposed, HIV-uninfected study participants. This sample size would provide high power to detect 75% reductions (hazard ratio of 0.24) from TB infection incidence rates in the control arm as low as 5.0% (see Table 5). If the reduction in the 96-week rate of TB infection or death due to INH prophylaxis is 50%, we still have satisfactory power if the failure rate in the control group is 15% or higher (see Table 6).

8.213 Breastfeeding and HIV transmission
It is anticipated that up to 15% of the HIV-infected mothers at these sites will breast feed (based on data available from these sites), which suggests that approximately 120 infants enrolled in the HIV-uninfected stratum out of the anticipated 800 will be breastfed. The majority of these women will breastfeed only intermittently after 6 weeks (the time of the first HIV DNA PCR). Breast milk transmission occurs in up to 13% of those cases associated with intermittent breast-feeding. This will lead to an additional 16 expected cases of transmission of HIV.

For the primary intention to treat analysis, these HIV-infected children will be included in the HIV-uninfected stratum and are expected to have the same or higher risk of TB infection as the truly HIV-uninfected infants. Including these study participants should only increase the power of analysis. Secondary analyses of the HIV-uninfected stratum will include study participants that remain HIV-uninfected for the duration of the study. The sample size of HIV-exposed, but uninfected children, allows for 680 evaluable study participants (15% loss to follow up), thus the exclusion of 16 study participants (2%) (who are HIV-infected, but enrolled as an uninfected study participants) will not lead to an under-powered secondary analysis.

Table 2: Power to detect differences in time to TB disease or death associated with a sample size of 500 (250 per arm) for HIV-infected study participants assuming 40.0% rate of TB disease or death over 96 weeks among control study participants and various event rates for the INH arm. Allows for 15% loss-to-follow-up and assumes two interim and one final analysis using O’Brien-Fleming type stopping boundaries.

<table>
<thead>
<tr>
<th>% with TB disease or death over 96 weeks among controls</th>
<th>% with TB disease or death over 96 weeks among INH recipients</th>
<th>% reduction in event rate due to INH prophylaxis</th>
<th>Power</th>
<th>Expected number of study participants with TB disease or death</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.0</td>
<td>25.0</td>
<td>37.5</td>
<td>91.8</td>
<td>130</td>
</tr>
<tr>
<td>40.0</td>
<td>27.5</td>
<td>31.3</td>
<td>78.6</td>
<td>138</td>
</tr>
<tr>
<td>40.0</td>
<td>30.0</td>
<td>25.0</td>
<td>57.3</td>
<td>146</td>
</tr>
<tr>
<td>40.0</td>
<td>32.5</td>
<td>18.8</td>
<td>36.2</td>
<td>152</td>
</tr>
<tr>
<td>% with TB disease or death over 96 weeks among controls</td>
<td>% with TB disease or death over 96 weeks among INH recipients</td>
<td>% reduction in event rate due to INH prophylaxis</td>
<td>Power</td>
<td>Expected number of study participants with TB disease or death</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>55.0</td>
<td>35.0</td>
<td>36.4</td>
<td>98.9</td>
<td>176</td>
</tr>
<tr>
<td>55.0</td>
<td>40.0</td>
<td>27.3</td>
<td>87.9</td>
<td>196</td>
</tr>
<tr>
<td>55.0</td>
<td>45.0</td>
<td>18.2</td>
<td>54.6</td>
<td>209</td>
</tr>
<tr>
<td>55.0</td>
<td>50.0</td>
<td>9.1</td>
<td>17.5</td>
<td>222</td>
</tr>
</tbody>
</table>

Table 3: Power to detect differences in time to TB disease or death associated with a sample size of 500 (250 per arm) for HIV-infected study participants assuming 55.0% rate of TB disease or death over 96 weeks among control study participants and various event rates for the INH arm. Allows for 15% loss-to-follow-up and assumes two interim and one final analysis using O’Brien-Fleming type stopping boundaries.

<table>
<thead>
<tr>
<th>% with TB disease or death over 96 weeks among controls</th>
<th>% with TB disease or death over 96 weeks among INH recipients</th>
<th>% reduction in event rate due to INH prophylaxis</th>
<th>Power</th>
<th>Expected number of study participants with TB disease or death</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>12.5</td>
<td>50.0</td>
<td>91.7</td>
<td>72</td>
</tr>
<tr>
<td>25.0</td>
<td>15.0</td>
<td>40.0</td>
<td>74.0</td>
<td>81</td>
</tr>
<tr>
<td>25.0</td>
<td>17.5</td>
<td>30.0</td>
<td>47.2</td>
<td>89</td>
</tr>
<tr>
<td>25.0</td>
<td>20.0</td>
<td>20.0</td>
<td>23.4</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 4: Power to detect differences in time to TB disease or death associated with a sample size of 500 (250 per arm) for HIV-infected study participants assuming 25.0% rate of TB disease or death over 96 weeks among control study participants and various event rates for the INH arm. Allows for 15% loss-to-follow-up and assumes two interim and one final analysis using O’Brien-Fleming type stopping boundaries.

<table>
<thead>
<tr>
<th>% with TB disease or death over 96 weeks among controls</th>
<th>% with TB disease or death over 96 weeks among INH recipients</th>
<th>% reduction in event rate due to INH prophylaxis</th>
<th>Power</th>
<th>Expected number of study participants with TB disease or death</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>12.5</td>
<td>50.0</td>
<td>91.7</td>
<td>72</td>
</tr>
<tr>
<td>25.0</td>
<td>15.0</td>
<td>40.0</td>
<td>74.0</td>
<td>81</td>
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<tr>
<td>25.0</td>
<td>17.5</td>
<td>30.0</td>
<td>47.2</td>
<td>89</td>
</tr>
<tr>
<td>25.0</td>
<td>20.0</td>
<td>20.0</td>
<td>23.4</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 5: Power to detect differences in 96-week rate of TB infection or death associated with a sample size of 800 (400 per arm) for perinatally-exposed, HIV-uninfected study participants assuming various rates of TB infection or death over 96 weeks among control subjects and a 75% reduction in failure rate due to INH prophylaxis. Allows for 15% loss-to-follow-up and assumes two interim and one final analysis using O’Brien-Fleming type stopping boundaries.
Table 6: Power to detect differences in 96-week rate of TB infection or death associated with a sample size of 800 (400 per arm) for perinatally-exposed, HIV-uninfected study participants assuming various rates of TB infection or death over 96 weeks among control subjects and a 50% reduction in failure rate due to INH prophylaxis. Allows for 15% loss-to-follow-up and assumes two interim and one final analysis using O'Brien-Fleming type stopping boundaries.

8.22 Accrual

After enrolling a few participants in December 2004, P1041 opened to unrestricted enrollment at the beginning of 2005. The HIV-uninfected stratum completed accrual with 806 participants (6 over target) on schedule on June 7, 2006. As of May 3, 2007, a total 237 out of the target 500 HIV-infected participants have been enrolled. Approximately 25 HIV-infected infants are being enrolled per month at the three South African sites and the team expects to reach target
accrual by third quarter 2008, or perhaps sooner with the enrollment of other interested international sites.

8.3 Data Analysis

8.3.1 Primary Analyses

Study participants will be classified according to their HIV infection status at study entry, and will be enrolled into a study stratum and randomly assigned a prophylaxis regimen. All primary, and most secondary, analyses will be performed on an intent-to-treat (ITT) basis; i.e., study participants will be analyzed according to their assigned study stratum and prophylaxis regimen, regardless of whether or not they become HIV-infected during the course of the study (i.e., due to breastfeeding) or whether or not they took their assigned prophylaxis regimen. Even prophylaxis discontinuation for study-mandated reasons do not warrant exclusion from these analyses, e.g., for exposure to household contacts with AFB smear-positive or culture-positive cases which would require appropriate prophylaxis per the South African TB control program. Analyses that are not ITT will be explicitly outlined below. Each stratum (HIV-infected or HIV-uninfected) will be analyzed separately.

TB disease will include possible, probable, and definite TB disease as defined in Appendix III for the primary analyses. TB infection will include both TB disease and positive TST defined in Appendix III for the primary analyses.

Kaplan-Meier plots will be used to describe the time to study endpoints, and hazard rates will be compared using two-sided log rank tests at the 0.05 level. For comparisons of hazard rates (except those to determine whether there is a residual effect of prophylaxis after prophylaxis discontinuation) events will be censored at 96 weeks after randomization.

Analyses of the residual effect of prophylaxis will not be according to the intent-to-treat principle. We will perform landmark analyses, including only study participants who remain endpoint free for 96 weeks.
of study follow-up. Hazard rates after 96 weeks on study will be compared using two-sided log rank tests at the 0.05 level.

Some of the secondary objectives use follow-up through 192 weeks, 96 weeks beyond the prophylaxis period, and will be analyzed on an ITT basis using all randomized study participants.

8.32 Secondary Analyses

In addition to the primary analyses, which follow the intent-to-treat principle, we will conduct secondary analyses on the subset of study participants who did not receive antiretroviral drugs during the first two years of the study using the same methods outlined above. Secondary analyses of the HIV-uninfected stratum will censor any endpoint at the time the child is last known to be HIV-uninfected. Secondary INH prophylaxis comparisons will also be made with endpoints censored at permanent discontinuation of study drug. We also will compare rates of endpoints by rates of adherence (e.g., comparing those taking greater than 365 days of study drug to those receiving 365 or fewer days of prophylaxis).

Secondary analyses including only definite and probable TB disease as defined in Appendix III in the definition of TB disease will also be conducted.

8.4 Data Safety and Monitoring Board (DSMB)

P1041 will be monitored by the DAIDS Therapeutics DSMB. The DSMB review will address at least the following:

- Efficacy of INH as described in the two primary objectives;
- Safety of INH;
- Current availability of ART for the study population as it relates to the conduct and analysis of P1041.

8.5 Data Monitoring

As noted above, the study will be reviewed by the DSMB for safety and efficacy. The first interim efficacy analyses of both strata were completed and
reviewed by the DSMB in June of 2006 and the second interim efficacy analysis will be conducted and reviewed by the DSMB one year after the first interim analysis, as originally planned. Due to the slower than expected accrual in the HIV-infected stratum, one or more additional interim analyses for efficacy for this stratum may be added in consultation with the DSMB.

Early stopping rules for efficacy will be guided by the O'Brien-Fleming symmetric group sequential boundaries. The Lan and DeMets implementation of the boundaries will be used to define proper nominal significance levels at the interim and final efficacy analyses (a total of 3 looks for the HIV-uninfected stratum and a total of 3 more looks for the HIV-infected stratum). Unless there are safety concerns, significant differences (as defined by the stopping boundaries) on primary efficacy endpoints will be required in order to terminate the study early.

Because of the concern that ART would decrease the rate of TB infection and disease in both randomized arms and would reduce the power of the study, the DSMB will monitor the rate of initiation of primary therapy for HIV prior to two years on-study treatment and the rates of TB infection and disease to determine whether any modification to the sample size is necessary.

In addition, monthly blinded safety monitoring reports will be sent to the medical officers, and protocol chairs and co-chairs for review which will include all Grade 3 and 4 toxicities and deaths.

Yearly, a report of the estimated rate of breastfeeding and of HIV-transmission among the HIV-uninfected study participants (as assessed initially by the results of the HIV DNA PCR done at screening) by site of enrollment will be provided to the medical officer and study chairs, co-chairs, and site principal investigators. This will allow the study team and/or site PI, to provide this information to policy makers as deemed appropriate by this group.

9.0 CLINICAL PHARMACOLOGY PLAN

9.1 Pharmacology Objectives:

9.11 Estimate the mean population PK parameters of INH including absorption rate constant (Ka), apparent plasma clearance (Cl/F), and apparent volume of
distribution (V/F) using a sparse sampling approach with non-linear mixed effect modeling (NONMEM).

9.12 Evaluate the allelic variation of N-acetyl acetyltransferase type 2 (NAT-2) genotype and determine its influence on the rate and extent of INH metabolism in study participants.

9.13 Explore the potential influence of INH rate of total body clearance and NAT-2 genotype on the outcome of INH chemoprophylaxis in study participants.

9.2 Pharmacology Studies

A sample size of 336 study participants will allow for 168 study participants on active drug to be sampled. Assuming a 10-15% drop out rate and accounting for 10-15% unevaluable samples (i.e. improper processing, shipping, storage, etc), two samples from each of 125 study participants on active drug will be quantifiable from blood collected for PK purposes at study weeks 0 or 12 and 84.

Population PK analysis is performed through sparse sampling of study drug levels. The initial goal in this approach is to estimate population parameters. There is no conventional procedure for calculation of sample size using this method, so calculation of a sample size to obtain a specified precision cannot be performed without using simulation, which requires accurate estimates of population parameters that are nonexistent for this population. Furthermore, we are interested in exploring the relationship between population PK and demographics (age, weight, gender, etc), HIV-status, and particularly NAT-2 genotype to determine whether they account for some of the variability in response; for the latter purpose including as many patients as possible is highly desirable. Based on literature consultation and discussions in feasibility with site personnel, protocol team, and population PK experts, 336 study participants is a reasonable number to obtain samples from. 250 samples (two sample timepoints per patient) will allow us to perform the proposed modeling technique with reasonable precision. This sample size should not only allow for the objectives of the pharmacology section to be met, but is large enough for estimation of individual study participant PK parameters after the initial population PK analysis has been completed (96-98).

Blood samples for determination of INH concentration will be obtained from 336 study participants enrolled in P1041 at either Cape Town or Durban. It is estimated that half (168) of these study participants will be receiving active INH. Study participants will be randomized to one of two groups and one of two arms (a total of 4 group/arm
combinations). Group membership will determine the study weeks at which PK sampling will be conducted and arm will indicate the hour post-dose of the blood draw: Group A will consist of study participants who will be sampled at week 0 (age 3-4 months) and week 84 (age 24-25 months). Group B will consist of study participants who will be sampled at week 12 (age 6-7 months) and week 84 (age 24-25 months of age). Each group will consist of 168 study participants and half (84) will be HIV-infected. The HIV-exposed uninfected cohort closed to accrual on June 7, 2006 under Version 1.0 of the protocol and no more HIV-exposed uninfected infants will be enrolled. Table 9 outlines the sampling weeks for the population PK analysis.

### Table 9: Sampling Times for Population Pharmacokinetic Analysis

<table>
<thead>
<tr>
<th>First Sampling Period</th>
<th>Study Participants</th>
<th>N</th>
<th>HIV-infected Study Participants (N/2)</th>
<th>Post-dose PK #1 Draw Time</th>
<th>Post-dose PK #2 Draw time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Study Week of PK Analysis</td>
<td>Study Participants Age (months)</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 1A</td>
<td>0</td>
<td>3-4</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 2A</td>
<td>0</td>
<td>3-4</td>
<td>84</td>
<td>42</td>
<td>2-hr</td>
</tr>
<tr>
<td>Group B</td>
<td>Study Week of PK Analysis</td>
<td>Study Participants Age (months)</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 1B</td>
<td>12</td>
<td>6-7</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 2B</td>
<td>12</td>
<td>6-7</td>
<td>84</td>
<td>42</td>
<td>2-hr</td>
</tr>
<tr>
<td>Second Sampling Period</td>
<td>Study Week of PK Analysis</td>
<td>Study Participants Age (months)</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Groups A &amp; B</td>
<td>Study Week of PK Analysis</td>
<td>Study Participants Age (months)</td>
<td>84</td>
<td>42</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 1A &amp; 1B</td>
<td>84</td>
<td>24-25</td>
<td>168</td>
<td>84</td>
<td>1-hr</td>
</tr>
<tr>
<td>Arm 2A &amp; 2B</td>
<td>84</td>
<td>24-25</td>
<td>168</td>
<td>84</td>
<td>2-hr</td>
</tr>
</tbody>
</table>

#### 9.21 Population Pharmacokinetic - Sampling

INH plasma concentrations will be determined in 336 study participants enrolled at Cape Town and Durban at study week 0 (Group A) or 12 (Group B) and again at week 84 (both Groups A and B). Each group will consist of 168 study participants and 84 of these will be on active INH. Following an observed dose of study drug, two blood samples will be collected for analysis at either 1- and 3-hr or 2- and 4-hr post dose depending on the arm assignment. See Table 9 for post-dose blood sampling schedules, total number of study participants, and certain sample population demographics.

#### 9.22 N-acetyl acetyltransferase type 2 (NAT-2) genotype
Cell pellets from PK sampling at week 0 (Group A) or 12 (Group B), and 84 (Groups A and B) will be processed and stored for future analysis. Although genotype analysis will be completed one time per study participant, the amount of DNA-yielding cells present in the blood samples is not pre-determinable. Therefore, cell pellets will be stored from both sampling periods to increase DNA-yielding cells. After study unblinding all study participants enrolled in the PK study, including those receiving INH placebo, will be genotyped. NAT-2 genotype will be used as a covariate in the NONMEM analysis to detect correlation to the rate of INH clearance among those on active drug. Study participants on INH placebo will serve as the control group.

9.3 Procedures for Pharmacologic Studies

9.3.1 Procedures for Population PK

Two venous blood samples will be obtained after an observed INH dose. If possible, study participants should not be fed one hour before and two hours after INH is administered. If possible, no other medication should be given on the same morning as the INH study. These drugs can be administered as soon as the last level has been obtained. If drugs have to be given during this period, the names of these drugs should be made known to the pharmacology laboratory when the specimens are submitted. The INH levels will be measured by methods described in Appendix VIII. The laboratory performing the INH levels will be unblinded as to the study participants’ randomization to INH or INH placebo so that only samples from participants actually receiving INH will be run.

The 336 study participants from Cape Town and Durban participating in the PK portion of the protocol will be randomly assigned to have samples drawn at either 3-4 months of age (Group A) or 6-7 months of age (Group B), and all of study participants will undergo additional PK sampling at 24-25 months of age. Two blood samples will be drawn on the day of PK sampling.

Data points (INH concentrations) for population PK analysis will be incorporated into NONMEM (Nonliner mixed-effect modeling) after each of the 336 study participants have completed the first period of PK sampling, and individual concentrations are available from the analytical laboratory.
9.32 Procedures for NAT-2 Genotyping

The cellular components from the Week 0 or 12 and 84 samples will be combined and stored for DNA extraction, amplification, and gel electrophoresis (see Appendix X for details).

9.4 Data Analysis

9.41 Population Pharmacokinetics

Using an experimental population PK design and NONMEM software, the following information will be determined from population PK modeling of plasma concentrations obtained using the above design: (1) an estimation of INH population PK parameters, and (2) description of variability of INH PK in this population. Sampling on more than one occasion is recommended when using NONMEM in order to estimate the components of variability. All data points from every study participant in the PK subset must be analyzed before application of this method.

Protocol pharmacologists will perform periodical assessments of available INH plasma concentrations beginning at the earliest time point possible once concentration data are available from the DMC. These assessments will provide ongoing verification that the objectives of the population PK analysis can be achieved using the current study design. If the pharmacologists observe a distribution of concentrations that are less than optimal for successful NONMEM analysis, the team will be notified and a protocol amendment to modify the PK study design will be submitted.

Standard PK models (one-compartment, first-order equations) will be fit to the concentration-time post-dose data with NONMEM to design a base model. The PK characteristics estimated for each drug include Ka, CL/F, and V/F.

The following covariate will be investigated using NONMEM: NAT-2 genotype, age, weight, HIV serostatus, gender, and TB diagnosis during INH chemoprophylaxis. Initially, the base model will be used to test each covariate individually, in a nested design. A reduction in objective function by >7.8 will indicate significance. Once all significant covariates are identified, a forward inclusion approach will be used to check for co-linearity.
After NONMEM analysis, additional PK parameters (i.e. Cmax and AUC) will be estimated using a Bayesian post-hoc computation for individual study participants. Based on PK parameter cut-offs, the distribution of slow, intermediate, and fast acetylators during infancy and at 2 years of age will be estimated.

9.43 NAT-2 Genotype

NAT-2 Genotypes include homozygous rapid (two rapid alleles), homozygous slow (two slow alleles), and heterozygous intermediate. Study participant NAT-2 genotype will be incorporated into NONMEM as a covariate. The percent of the population during infancy and at two years of age whose genotype and phenotype are concordant will be determined. The influence of NAT-2 genotype on the outcome of INH chemoprophylaxis will also be explored, with study participants receiving placebo INH serving as the control group.

9.44 Anticipated Outcomes

Following NONMEM analysis, the systemic exposure to INH in P1041 subjects will be compared to existing adult and pediatric mean values to evaluate whether the P1041 INH dose produced similar systemic exposure within this patient population during infancy and at 2 years of age.

On the basis of this preliminary information, a discrepancy between acetylation genotype and phenotype in children may be seen, depending on the age of the child. The population based PK analysis in this protocol will address hepatic NAT-2 enzyme maturation rates within this pediatric population.
10.0 HUMAN SUBJECTS

The Division of AIDS has concluded that this protocol does not meet Federal requirements governing prisoner participation in clinical trials and should not be considered by local IRBs for the recruitment of prisoners.

Each site which receives US HHS funding and follows the United States Code of Federal Regulations Title 45 – Public Welfare, Part 46 – Protection of Human Subjects (also known as the Common Rule) should have on record at the site a plan that detects and addresses any change in guardianship occurring in pediatric subjects and determines when a study subject must have a consent process which involves a legally authorized representative (LAR) other than a family member with guardianship. The plan will include how the site determines when a LAR is initially or no longer needed and how frequently the LAR resigns the consent. The plan should follow all IRB, local, and state guidelines. Confirmation of such a plan at a site should be submitted with protocol registration materials.

10.1 Ethics Committee Review and Informed Consent

This protocol, the informed consent documents (Appendix XIII-A and XIII-B), and any subsequent modifications must be reviewed and approved by the Ethics Committee responsible for oversight of the study. Written informed consent must be obtained from the parents or legal guardians of study participants. The informed consent will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the parent or legal guardian of the study participant.

10.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified only by a coded number to maintain subject confidentiality. All records will be kept in a secured area. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by NIAID, the South African Medicines Control Council (MCC), the South African Ethics Committee, or the Office for Human Research Protection (OHRP).
10.3 Study Discontinuation

The study may be discontinued at any time by NIAID, the country specific governing bodies and Ethics Committees, or other government agencies as part of their duties to ensure that research subjects are protected.

10.4 Regulatory Authorities

This protocol will be carried out under the provisions of Good Clinical Practice (GCP) Guidelines. Additionally, the trial will be conducted in full accordance with the principles of the Declaration of Helsinki, September 1989 and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, May 1997, and the Guidelines for Good Practice in the conduct of Clinical Trials in Human Participants (Section 9.0)

11.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by PACTG policies and in accordance with the Memorandum of Agreement between the South African Paediatric Infectious Diseases Research Unit of the WITS Health Consortium and the PACTG. Any presentation, abstract, or manuscript will be made available for review by the Secure the Future Foundation prior to submission.

12.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other bloodborne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently legislated by the South African Hazardous Biological Substances Occupational Health and Safety (Government Gazette Vol 438, No 22956, Dec 2001) or country specific guidelines.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the International Air Transport Association Dangerous Goods Regulations-Packing Instruction 602. Refer to individual carrier guidelines (e.g., Federal Express or Airborne) for specific instructions.
13.0 REFERENCES


REFERENCES (Cont.)


21. Personal communication between Drs. Mark Cotton and Peter Donald.


REFERENCES (Cont.)


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91. Schaaf HS, Seifart HI, Parkin DP, Donald PR. The clinical pharmacokinetics of isoniazid (INH) in children. 33rd World Conference on Lung Health of the IUATLD, October 6-10, 2002, Montreal, Canada.


## SCHEDULE OF EVALUATIONS FOR HIV-INFECTED STUDY PARTICIPANTS

### Clinical Evaluations

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1. Parent/legal guardian may sign the informed consent as early as the first day of life of the study participant.
2. Study participants must receive BCG vaccine at least 90 days prior to INH/placebo initiation.
3. Physical exam (height, weight, vital signs, symptoms, HIV infection status assessment, assessment of presence/absence of BCG skin reaction). The Algorithm to Diagnose Clinical Tuberculosis (Appendix II) is to be used to screen for pulmonary TB at each study visit.
4. Liver Function Tests (LFTs)—Serum Glutamic Oxalacetic Transaminase [(SGOT) Aspartate Amino Transferase (AST)] and Serum Glutamate Pyruvate Transaminase [(SGPT) Amino Alanine Transferase (ALT)]. Subjects who continue on PCP prophylaxis after their first year of life will have LFTs done every three months until TPM/SMX is discontinued. All other subjects will have LFTs done every six months after the first year of life until INH/placebo is discontinued. LFTs will be done after study visit week 96 only if abnormal at the previous visit.
5. Study participants will return to the clinic within 48-72 hours of TST/PPD tests to determine results and course of action.
6. See Appendices V and VI.
7. Adherence will be measured every 12 weeks to study visit week 96 (or at least three times a year). If study participant is on PCP prophylaxis after week 96, adherence will be measured every 24 weeks to study visit 192 (or at least two times a year).
8. Only done at Johannesburg.
9. Hematology (complete blood count, cell differential, platelet count). Study participants who continue PCP prophylaxis after the first year of life will have CBC performed every three months until PCP prophylaxis is discontinued.
10. Obtain a 3.5-mL volume of blood collected in EDTA. The CD4 cell count and hematology survey will be determined on a portion of this sample and plasma will be prepared from an additional portion of this sample for HIV RNA PCR and HIV EIA (see Appendix VII). When HIV DNA PCR is required, obtain a 1.0 mL volume of blood (for pre-entry sample) or 2.0 mL volume of blood (for later sample) collected in EDTA and use the same sample for HIV DNA PCR and hematology.
11. Tests should be run within two weeks. Store at least 4 pellets for re-testing (See Appendix VII). The pre-entry DNA PCR must be performed in a laboratory that has been certified by the Virology Quality Assurance (VQA) using the Roche Amplicor HIV DNA test version 1.5. If a DNA PCR was obtained as part of another study or for patient care, it may be used as the pre-entry sample provided it was obtained from the subject > 28 days of life and up to 120 days of life, and in a PACTG VQA certified laboratory. The DNA PCR sample does not have to be repeated prior to entry, but the results of the pre-entry DNA PCR MUST be available prior to study entry.
12. Tests should be run semi-real or real time (See Appendix VII).
13. HIV RNA determination (done in a VQA certified laboratory) at study entry will be used as confirmation of the initial positive HIV DNA PCR measured at pre-entry. If study entry visit HIV RNA determination and the one obtained at the week 12 visit are both below detectable levels, a repeat HIV DNA PCR will be done at the 24 week visit and the pre-entry HIV DNA sample will be re-tested.
14. Performed at week 60 visit for subjects with positive HIV DNA PCR at pre-entry, but with subsequent negative results on all HIV DNA and RNA PCR assays. Subjects who are breastfed beyond week 60 should have an HIV-1 EIA performed 6 to 12 weeks following the cessation of breastfeeding.

15. Population PK analysis will be done at Cape Town and Durban in a subset of 336 study participations. Blood will be drawn at study entry or week 12 at 1hr and 3hr (Group/Arm 1A and 2A) or 2hr and 4hr (Group/Arm 1B and 2B) post observed INH dose. Blood samples will also be taken at study week 84 at 1hr and 3hr (Group/Arm 1A and 2A) or 2hr and 4hr (Group/Arm 1B and 2B) post observed INH dose. Two samples of 3-mL aliquot each (to increase DNA-yielding cells for NAT-2 genotype analysis) will be obtained at week 84. Cell pellets for NAT-2 Genotype analysis will be stored from study entry or week 12 and at week 84 for NAT-2 genotype analysis.

16. INH/Placebo CANNOT be started before 91 days of age and there must be at least 90 days between BCG vaccine and start of INH/placebo.

17. TMP-SMX will be the first-line agent for PCP prophylaxis. In case of toxicity, alternative agents should be chosen according to section 6.2.

18. PCP prophylaxis should be continued for all HIV-infected study participants during the first year of life and for all HIV-uninfected study participants during the 1st year of life while breastfeeding.

19. PCP prophylaxis should be continued after the first year of life according to country specific guidelines.

20. Pre-entry visit could occur as early as ≥28 days of age and as late as ≤120 days of age. Pre-entry and entry visits should be performed on consecutive days.

21. Study entry visit or Day 0 could be scheduled as early as 91 days of age and no later than 120 days of age. DNA PCR from Pre-Entry visit must be available prior to Study Entry. There must be a window of at least 90 days between of BCG and start of INH prophylaxis/placebo.

22. +/- 6 weeks for study visit weeks 12-96; +/- 8 weeks for study visit weeks 108-192.
# APPENDIX I-B

## SCHEDULE OF EVALUATIONS FOR HIV-UNINFECTED STUDY PARTICIPANTS

<table>
<thead>
<tr>
<th>VISIT SCHEDULE</th>
<th>Pre-Entry</th>
<th>Study Entry</th>
<th>12-Weeks</th>
<th>24-Weeks</th>
<th>36-Weeks</th>
<th>48-Weeks</th>
<th>60-Weeks</th>
<th>72-Weeks</th>
<th>84-Weeks</th>
<th>96-Weeks</th>
<th>120-Weeks</th>
<th>144-Weeks</th>
<th>168-Weeks</th>
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<td>Total Blood Volume</td>
<td>3.0ml 1.0ml 1.0ml 2.0ml 3.0ml 1.0ml 2.0ml 0ml 1.0ml 2.0ml 1.0ml 2.0ml 1.0ml 2.0ml</td>
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</table>

*Obtain q 12 weeks until PCP prophylaxis is discontinued.*
APPENDIX I-B (Cont.)

<table>
<thead>
<tr>
<th>VISIT SCHEDULE</th>
<th>Pre-Entry 15</th>
<th>Study Entry 16</th>
<th>12-Weeks 17</th>
<th>24-Weeks 17</th>
<th>36-Weeks 17</th>
<th>48-Weeks 17</th>
<th>60-Weeks 17</th>
<th>72-Weeks 17</th>
<th>84-Weeks 17</th>
<th>96-Weeks 17</th>
<th>120-Weeks 17</th>
<th>144-Weeks 17</th>
<th>168-Weeks 17</th>
<th>192-Weeks 17</th>
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</thead>
<tbody>
<tr>
<td>Pharmacology</td>
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<tr>
<td>Population PK and NAT-2 Genotyping 12</td>
<td>4 ml 12</td>
<td>4 ml 12</td>
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<td></td>
</tr>
<tr>
<td>INH/Placebo 13</td>
<td>Start</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>Stop</td>
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</tr>
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<td>PCP 14</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Max. Blood (ml)</td>
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<td>5.0ml</td>
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<td>3.0ml</td>
<td>1.0ml</td>
<td>2.0ml</td>
<td>0ml</td>
<td>1.0ml</td>
<td>2.0ml</td>
<td>1.0ml</td>
<td>2.0ml</td>
<td>1.0ml</td>
<td>2.0ml</td>
</tr>
</tbody>
</table>

1. Parent/legal guardian may sign the informed consent form as early as the first day of life of the study participant.
2. Study participants must receive BCG vaccine at least 90 days prior to INH/placebo initiation.
3. Physical exam (height, weight, vital signs, symptoms, HIV infection status, **assessment of presence/absence of BCG skin reaction**). The Algorithm to Diagnose Clinical Tuberculosis (Appendix II) is to be used to screen for pulmonary TB at each study visit.
4. Liver Function Tests (LFTs)--Serum Glutamic Oxalacetic Transaminase [(SGOT) Aspartate Amino Transferase (AST)] and Serum Glutamate Pyruvate Transaminase [(SGPT) Amino Alanine Transferase (ALT)]. LFTs will be done every 12 weeks for those on PCP prophylaxis and every 24 weeks for those not on PCP prophylaxis until INH/placebo is discontinued. LFTs will be performed after study visit week 96 only if abnormal at the previous visit.
5. Subjects will return to the clinic within 48-72 hours of TST/PPD tests to determine results and course of action.
6. See Appendices V and VI.
7. Adherence will be measured every 12 weeks to study visit week 96 (or at least 3 times per year).
8. Only done at Johannesburg.
9. All study participants with negative HIV DNA PCR at pre-entry and positive DNA PCR study visit week 24 will have a third DNA PCR at study visit week 36. If DNA PCRs at the weeks 24 and 36 are positive, the study participant should be considered to be HIV-infected and should be followed as per Appendix I-A. If the week 24 specimen is positive and the week 36 specimen is negative, an additional specimen should be obtained as soon as possible and additional pellets from all 4 specimens should be processed and tested. Any infants of indeterminate HIV infection status should be reported to the protocol team.

10. Tests should be run within two weeks. Store at least 4 pellets for re-testing (See Appendix VII)

11. HIV-exposed but uninfected children (as per the pre-entry HIV DNA PCR) will have an HIV-1 EIA at the 36 Week Visit. Subjects with a positive result on the 36 Week EIA will have a repeat EIA done at the 60 Week visit. Additionally, subjects who have been breastfed beyond the 24 Week visit will have a repeat HIV-1 EIA performed at the 60 Week Visit. Any subject with a positive result on the 60 Week visit EIA will be considered to be HIV-infected and should be followed as per Appendix IA.

12. Population PK analysis will be done at Cape Town and Durban in a subset of 336 study participants. Cell pellets for NAT-2 Genotype analysis will be stored from study entry or week 12 and at week 84 for NAT-2 genotype analysis. Two samples of 2 mL aliquot each will be drawn at entry or week 12 at 1hr and 3hr (Group/Arm 1A and 2A) or 2hr and 4hr (Group/Arm 1B and 2B) post-observed INH dose. Blood samples will also be taken at study week 84 at 1hr and 3hr (Group/Arm 1A and 2A) or 2hr and 4hr (Group/Arm 1B and 2B) post-observed INH dose. Two samples of 3-mL aliquot each (to increase DNA-yielding cells for NAT-2 genotype analysis) will be obtained.

13. INH/placebo CANNOT be started before 91 days of age and there must be at least 90 days between BCG vaccine and start of INH/placebo.

14. TMP/SMX prophylaxis will be given at least until a repeat HIV DNA PCR is performed at 6 months of age; following confirmation of the child being HIV-1 uninfected at 6 months of age, TMP/SMX prophylaxis will be stopped in children not being breast-fed but continued in those still being breast-fed until HIV infection status has been determined based on EIA at the 60-week study visit.

15. Pre-entry visit could be scheduled as early as >28 days of age and as late as ≤120 days of age.

16. Study entry visit or Day 0 could be scheduled as early as 91 days of age and no later than 120 days of age. DNA PCR from pre-entry visit MUST be available prior to study entry.

17. +/- 6 weeks for study visit weeks 12-96; +/- 8 weeks for study visit weeks 120-192.
### APPENDIX II

**ALGORITHM TO SCREEN FOR AND DIAGNOSE CLINICAL TUBERCULOSIS**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks of illness (including cough)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Nutrition (% weight for age)/malnutrition(^1)</td>
<td>&lt;80%</td>
</tr>
<tr>
<td></td>
<td>60-80%</td>
</tr>
<tr>
<td></td>
<td>&lt;60%/drop≥ 2 lines</td>
</tr>
<tr>
<td>Family history of TB</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Reported by family</td>
</tr>
<tr>
<td></td>
<td>Sputum proven</td>
</tr>
<tr>
<td>Tuberculin skin test(^2)</td>
<td>Reactive (≥5 or ≥10 mm)(^3)</td>
</tr>
<tr>
<td>Fever not responding to rx for &gt;2 weeks</td>
<td>X</td>
</tr>
<tr>
<td>Confirmed or suspected EPTB (as per definition in Appendix III)</td>
<td>X</td>
</tr>
</tbody>
</table>

1. Evaluation for malnutrition will be done per WHO guidelines outlined in Management of Severe Malnutrition- 1999 (http://www.who.int/nut/documents/manage_severe_malnutrition_eng.pdf). This guide provides assessment tools for the evaluation of malnutrition based on z-scores, and clinical and laboratory evaluations.

2. A diameter of ≥ 5mm in HIV-infected children, and ≥ 10mm in uninfected children will be considered as reactive.

A study participant with an abnormal chest x-ray suggestive of PTB (See Appendix III) and who scores ≥6 will be diagnosed as possible clinical PTB.

Extra-thoracic lymphadenopathy, joint/bone involvement, abdominal mass, meningitis and TB spine will be diagnosed by EPTB criteria (See Appendix III).

Study participants who score ≥4 during routine screening (excluding TST) will be screened further by TST and chest x-ray. This algorithm, excluding TST, is to be used to screen for pulmonary TB at each study visit and study participants with a score of greater than or equal to 4 on routine screening will be further screened by TST and chest x-ray. If the chest x-ray is suggestive of pulmonary TB and the algorithm score is greater than or equal to 6, a diagnosis of possible pulmonary TB is to be made (as per Appendix III).
APPENDIX III

TUBERCULOSIS-RELATED TERMINOLOGY USED IN P1041

I – DEFINITIONS

A) **TB infection**: Any manifestation of infection by *Mycobacterium tuberculosis*.

B) **Latent TB infection**: Subset of TB infection that refers to a positive tuberculin skin test in the absence of any clinical, radiographic, or laboratory evidence of disease caused by *Mycobacterium tuberculosis*.

C) **TB disease**: Subset of TB infection that refers to the presence of clinical, radiographic, or laboratory evidence of disease caused by *Mycobacterium tuberculosis*.

Schematic:

```
   TB infection
     /\
    /  \
  Latent TB Mantoux +ve  TB disease Clinical and/or radiographical and/or laboratory
```

D) **TB Exposure**: Contact with any smear or culture-positive case who has not received treatment or is still on treatment.

II – CATEGORIZATION OF TB DISEASE

A) **Definite Mycobacterium tuberculosis**: *Mycobacterium tuberculosis (M.tb.)* isolated from any of the following: gastric washings, induced sputum or other sterile body fluid or tissue or positive stain on CSF. Samples from induced sputum and gastric washings can be combined and processed as one sample.

B) **Probable Pulmonary TB (PTB)**: Positive AFB (auramine fluorochrome, most commonly used in South Africa, or Ziehl Neelson (ZN)) stain on a specimen obtained by gastric washings or induced sputum in a child who fulfills at least one of the following:
APPENDIX III (Cont.)

1. Presence of at least 2 clinical criteria:
   - Cough > 2 weeks duration
   - Family history of PTB in the prior 24 weeks
   - Reactive TST (≥5 mm in an HIV +, ≥10 mm in HIV-)
     - Weight <3rd percentile for age or a decrease in weight that has crossed 2 major growth percentiles since the last documented weight
   - Fever of unknown origin >2 weeks duration

OR:

2. Abnormal chest x-ray with at least one of the following:
   - Hilar lymphadenopathy
   - Paratracheal lymphadenopathy
   - Alveolar consolidation
   - Miliary pattern
   - Lung parenchymal breakdown/cavitation
   - Ghon focus

C) Possible PTB:

While the presence of a reactive TST (≥5mm in HIV-infected, ≥10mm in HIV-uninfected) will be used to support the diagnosis of possible pulmonary TB, because of the appreciable rate of false negative TST among children, the absence of a positive TST will not negate this diagnosis if the child has:

1. a CXR suggestive of PTB, and
2. a score of ≥6 on the attached algorithm (See Appendix II).

Thus a child with a negative TST could be considered to have Possible PTB if they have the characteristic CXR findings plus fever for >2 weeks, significant malnutrition, > 4 weeks of illness, and a family history of TB (sputum proven), and a negative microbiology (stain or culture).

The Algorithm to Diagnose Clinical Tuberculosis (Appendix II) is to be used to screen for pulmonary TB at each study visit. Study participants who score greater than or equal to 4 on
the algorithm during routine screening (excluding TST) will be screened further with TST and a chest X-ray (CXR).

APPENDIX III (Cont.)

A child will be considered to have possible PTB if the child has an abnormal CXR suggestive of PTB and at least one of the following findings:

- Hilary lymphadenopathy
- Paratracheal lymphadenopathy
- Alveolar consolidation
- Miliary pattern
- Lung parenchymal breakdown/cavitation
- Ghon focus (poorly defined opacity often near pleural surface 1-2 cm in diameter; may be single or multiple)

AND one of the following:

- Reactive TST (≥ 5mm in HIV+, ≥10mm in HIV-); or
- Scores ≥ 6 (See Appendix II)

Note: Extra-thoracic lymphadenopathy, joint/bone involvement/abdominal mass/meningitis and TB spine will be diagnosed as per extrapulmonary TB criteria.

D) Probable Extrapulmonary TB (EPTB):

1. TB Meningitis: (Any child with a positive CSF stain for AFB will be considered to have definite TB meningitis).
   Abnormal neurological evaluation, CSF stain negative for AFB, and at least one of the following clinically correlated findings:

   - Family history of PTB in the prior 24 weeks
   - Reactive TST (≥ 5 mm in HIV +, ≥ 10 mm in HIV-)
   - CXR suggestive of PTB
APPENDIX III (Cont.)

AND one of the following:

- Radiologic evidence of TB on CT of the brain (Tuberculoma, hydrocephalus or basal enhancement)
- Biologic evidence of TB meningitis:
  - CSF total protein > 0.6 mg/dL, or
  - Leukocytosis with a lymphocyte predominance and the absence of other pathogens identified

2. Abdominal TB
   At least one of the following clinically correlated findings:
   - Family history of PTB in the prior 24 weeks
   - Reactive TST (≥ 5 mm in an HIV+, ≥ 10 mm in HIV-)
   - CXR suggestive of PTB
   AND at least one of the following:
   - Abnormal ultrasound showing matted lymph nodes or matted small bowel
   - CT of the abdomen showing matted lymph nodes or matted small bowel or ascites.
   - Pathological/histopathologic evaluation consistent with TB

3. Extrathoracic TB lymphadenopathy

Note: Biopsy sample should always be submitted for mycobacterial & fungal & bacterial culture.

Presence of an enlarged lymph node at least one of the following clinically correlated findings:

- Family history of PTB in the prior 24 weeks
- Reactive TST (≥ 5 mm in an HIV-infected, ≥ 10 mm in HIV-negative)
- CXR suggestive of PTB
APPENDIX III (Cont.)

AND one of the following:

- Typical histopathology seen on biopsy (granuloma)
- Pathologic evaluation consistent with inflammatory reaction (in an HIV-infected subject)

4. TB Pericarditis:
Presence of at least one of the following clinically correlated findings:

- Family history of PTB in the prior 24 weeks
- Reactive TST ($\geq 5$ mm in an HIV-infected, $\geq 10$ mm in HIV-uninfected)
- CXR suggestive of PTB

AND one of the following:

- Abnormal echocardiography of the heart (constrictive pericarditis or pericardial effusion).
- If pericardiocentesis done: no other pathogens identified.
- Typical histopathology (granuloma), or no other pathogens are identified.

5. TB of the Bone/Joint:
Presence of at least one of the following clinical correlation findings:

- Family history of PTB in the prior 24 weeks
- Reactive TST ($\geq 5$ mm in an HIV-infected, $\geq 10$ mm in HIV-uninfected)
- CXR suggestive of PTB

AND one of the following:

- X-ray of the long bone showing changes consistent with TB such as erosion of the epiphysis or metaphysis.
- If bone aspirate sent, typical histopathology (granuloma), or no other pathogens are identified.

6. TB Spine:
X-ray evidence of collapse of a vertebral body.
All aspirates must be sent for appropriate culture; on AFB positive specimens, possibly should include diagnostic PCR in algorithm.

APPENDIX III (Cont.)

7. Disseminated vs. Miliary TB

Tubercle bacilli from the lymphadenitis of the primary complex in the lung probably are disseminated during the incubation period in all cases of TB infection. The clinical picture produced by the lymphohematogenous spread is determined by the host susceptibility at the time of spread and by the quantity of tubercle bacilli released. Three clinical forms can be recognized. These are as follows:

1. Occult which usually remains so although it may be occult initially with metastatic, extra pulmonary or extra thoracic lesions appearing months or years later. While the extra thoracic complications represent systemic spread they should not be considered to represent disseminated disease unless there are 2 or more foci occurring simultaneously.

2. Protracted hematogenous TB, a rare entity (at least in the HIV-uninfected child) characterized by high, spiking fever, marked leukocytosis, hepatosplenomegaly, and generalized lymphadenopathy. The frequency of this entity is so low that it is not uniformly reported in the majority of published series.

3. Miliary TB secondary to the discharge of a caseous focus into a blood vessel usually occurring 2-6 months after primary infection in infancy. Clinically, miliary TB can be identified by the finding of multiple, diffuse tubercles (reticular-nodular infiltrates) on chest x-ray.

In summary, disseminated TB can be defined as either the occurrence of extrathoracic infectious foci (eg., TB meningitis, abdominal TB, etc), and/or the occurrence of protracted hematogenous TB. Miliary TB is a unique form of disseminated disease, hence the separate definition.
# APPENDIX IV-A

## DIVISION OF AIDS

**TOXICITY TABLE for GRADING SEVERITY of PEDIATRIC (≤ 3 MONTHS OF AGE) ADVERSE EXPERIENCES)**

April 1994

For other findings, the Toxicity Table for children > 3 months of age (September, 1993) is applicable. All values here are for term newborns. Preterm infants should be judged by a comparison of local normal ranges and the newborn ranges identified here.

<table>
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<th>PARAMETER</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
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<tr>
<td><strong>HEMATOLOGY</strong></td>
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<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 days old</td>
<td>13.0-14.0</td>
<td>12.0-12.9</td>
<td>&lt;12</td>
<td>Cardiac Failure 2ndary to Anemia</td>
</tr>
<tr>
<td>8-21 days old</td>
<td>12.0-13.0</td>
<td>10.0-11.9</td>
<td>&lt;10.0</td>
<td>Cardiac Failure 2ndary to Anemia</td>
</tr>
<tr>
<td>22-35 days old</td>
<td>9.5-10.5</td>
<td>8.0-9.4</td>
<td>&lt;8.0</td>
<td>Cardiac Failure 2ndary to Anemia</td>
</tr>
<tr>
<td>36-56 days old</td>
<td>8.5-9.4</td>
<td>7.0-8.4</td>
<td>&lt;7.0</td>
<td>Cardiac Failure 2ndary to Anemia</td>
</tr>
<tr>
<td>57-90 days old</td>
<td>9.0-9.9</td>
<td>7.0-8.9</td>
<td>&lt;7.0</td>
<td>Cardiac Failure 2ndary to Anemia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abs Neutrophil Ct</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day old</td>
<td>5000-7000</td>
<td>3000-4999</td>
<td>1500-2999</td>
<td>&lt;1500</td>
</tr>
<tr>
<td>2-7 days old</td>
<td>1750-2500</td>
<td>1250-1749</td>
<td>750-1249</td>
<td>&lt;750</td>
</tr>
<tr>
<td>8-56 days old</td>
<td>1200-1800</td>
<td>900-1199</td>
<td>500-899</td>
<td>&lt;500</td>
</tr>
<tr>
<td>57-90 days old</td>
<td>750-1200</td>
<td>400-749</td>
<td>250-399</td>
<td>&lt;250</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bilirubin</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 days old</td>
<td>20-25</td>
<td>26-30</td>
<td>&gt;30</td>
<td></td>
</tr>
<tr>
<td>7-60 days old</td>
<td>1.1-1.9xN</td>
<td>2.0-2.9xN</td>
<td>3.0-7.5xN</td>
<td>&gt;7.5xN</td>
</tr>
<tr>
<td>61-90 days old</td>
<td>1.1-1.9xN</td>
<td>2.0-2.9xN</td>
<td>3.0-7.5xN</td>
<td>&gt;7.5xN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Creatinine</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 days old</td>
<td>1.0-1.7</td>
<td>1.8-2.4</td>
<td>2.5-3.0</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>7-60 days old</td>
<td>0.5-0.9</td>
<td>1.0-1.4</td>
<td>1.5-2.0</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>61-90 days old</td>
<td>0.6-0.8</td>
<td>0.9-1.1</td>
<td>1.2-1.5</td>
<td>&gt;1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cr Clearance</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 days old</td>
<td>35-40</td>
<td>30-34</td>
<td>25-29</td>
<td>&lt;25</td>
</tr>
<tr>
<td>7-60 days old</td>
<td>45-50</td>
<td>40-44</td>
<td>35-39</td>
<td>&lt;35</td>
</tr>
<tr>
<td>61-90 days old</td>
<td>60-75</td>
<td>50-59</td>
<td>35-49</td>
<td>&lt;35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low Calcium</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 days old</td>
<td>6.5-6.9</td>
<td>6.0-6.4</td>
<td>5.5-5.9</td>
<td>&lt;5.5</td>
</tr>
<tr>
<td>7-60 days old</td>
<td>7.6-8.0</td>
<td>7.0-7.5</td>
<td>6.0-6.9</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>61-90 days old</td>
<td>7.8-8.4</td>
<td>7.0-7.7</td>
<td>6.0-6.9</td>
<td>&lt;6.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Calcium</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 days old</td>
<td>12.0-12.4</td>
<td>12.5-12.9</td>
<td>13.0-13.5</td>
<td>&gt;13.5</td>
</tr>
<tr>
<td>7-60 days old</td>
<td>10.5-11.2</td>
<td>11.3-11.9</td>
<td>12.0-13.0</td>
<td>&gt;13.0</td>
</tr>
<tr>
<td>61-90 days old</td>
<td>10.5-11.2</td>
<td>11.3-11.9</td>
<td>12.0-12.9</td>
<td>&gt;= 13.0</td>
</tr>
</tbody>
</table>
### DIVISION OF AIDS

**TOXICITY TABLE for GRADING SEVERITY of PEDIATRIC (> 3 MONTHS OF AGE) ADVERSE EXPERIENCES)**

April 1994

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HEMATOLOGY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin &gt; 3 mo.- &lt; 2 y.o.</td>
<td>9.0-9.9</td>
<td>7.0-8.9</td>
<td>&lt;7.0</td>
<td>Cardiac Failure secondary to anemia</td>
</tr>
<tr>
<td>Hemoglobin &gt; = 2 y.o.</td>
<td>10-10.9</td>
<td>7.0-9.9</td>
<td>&lt;7.0</td>
<td>Cardiac Failure secondary to anemia</td>
</tr>
<tr>
<td>Abs Neutrophil Ct</td>
<td>750-1200</td>
<td>400-749</td>
<td>250-399</td>
<td>&lt;250</td>
</tr>
<tr>
<td>Platelets</td>
<td>50,000-75,000</td>
<td>25,000-49,999</td>
<td>&lt;25,000 or bleeding</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>1.1-1.25xN</td>
<td>1.26-1.5xN</td>
<td>1.51-3.0xN</td>
<td>&gt;3xN</td>
</tr>
<tr>
<td>PTT</td>
<td>1.1-1.66xN</td>
<td>1.67-2.33xN</td>
<td>2.34-3.0xN</td>
<td>&gt;3xN</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.1-1.9xN</td>
<td>2.0-2.9xN</td>
<td>3.0-7.5xN</td>
<td>&gt;7.5xN</td>
</tr>
<tr>
<td>AST (SGOT)</td>
<td>1.1-4.9xN</td>
<td>5.0-9.9xN</td>
<td>10.0-15.0xN</td>
<td>&gt;15.0xN</td>
</tr>
<tr>
<td>ALT (SGPT)</td>
<td>1.1-4.9xN</td>
<td>5.0-9.9xN</td>
<td>10.0-15.0xN</td>
<td>&gt;15.0xN</td>
</tr>
<tr>
<td>GGT</td>
<td>1.1-4.9xN</td>
<td>5.0-9.9xN</td>
<td>10.0-15.0xN</td>
<td>&gt;15.0xN</td>
</tr>
<tr>
<td>Pancreatic Amylase</td>
<td>1.1-1.4xN</td>
<td>1.5-1.9xN</td>
<td>2.0-3.0xN</td>
<td>&gt;3.0xN</td>
</tr>
<tr>
<td>Total Amylase + Lipase*</td>
<td>1.1-1.4xN</td>
<td>1.5-2.4xN</td>
<td>2.5-5.0xN</td>
<td>&gt;5.0xN</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>7.5-9.9</td>
<td>10-12.4</td>
<td>12.5-15.0</td>
<td>&gt;15.0 or Gout</td>
</tr>
<tr>
<td>CPK</td>
<td>See Neuromuscular Toxicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>Mild</td>
<td>Moderate-No Rx Needed</td>
<td>Moderate-Rx Needed</td>
<td>Severe-Hospital and Rx</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Soft stools</td>
<td>Liquid stools</td>
<td>Liquid stools &amp; Mild Dehydration Bloody stools</td>
<td>Dehydration requiring IV therapy or Hypotensive Shock</td>
</tr>
<tr>
<td>Constipation</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Distention and Vomiting</td>
</tr>
<tr>
<td>Nausea</td>
<td>Mild</td>
<td>Moderate-Decreased po intake</td>
<td>Severe-Little po intake</td>
<td>Unable to ingest food or fluid for &gt;24 hours</td>
</tr>
<tr>
<td>Vomiting</td>
<td>&lt;1 episode/day</td>
<td>1-3 episodes/day or duration &gt;3d</td>
<td>&gt;3 episodes/day or duration &gt;7d</td>
<td>Intractable Vomiting</td>
</tr>
</tbody>
</table>

Comments:

*Both amylase and lipase must be elevated to the same grade or higher (i.e. if total amylase is Grade 4, but lipase is only Grade 1, the Toxicity Grade is 1. In pediatric HIV patients, the most common source of serum amylase is the salivary glands. Salivary amylase elevations are generally not clinically significant. When amylase is released from damaged pancreatic cells, it can be a marker of pancreatitis. In most cases of clinical pancreatitis, lipase will also be elevated. However, lipase is also a non-specific marker. Combined elevation of amylase and lipase (each >5 x normal) often indicates pancreatic disease and requires evaluation. However, in the absence of pancreatic disease, drug can be resumed even at Grade 3 and 4 toxicities.*
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CREATININE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Month-2 Years</td>
<td>0.6-0.8</td>
<td>0.9-1.1</td>
<td>1.2-1.5</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>2 Years-Adolescent</td>
<td>0.7-1.0</td>
<td>1.1-1.6</td>
<td>1.7-2.0</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>Adolescents</td>
<td>1.0-1.7</td>
<td>1.8-2.4</td>
<td>2.5-3.5</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>Creatinine Clearance</td>
<td>60-75 cc/min/1.73 m²</td>
<td>50-59 cc/min/1.73 m²</td>
<td>35-49 cc/min/1.73 m²</td>
<td>&lt;35 cc/min/1.73 m²</td>
</tr>
<tr>
<td><strong>ELECTROLYTES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Sodium</td>
<td>145-149</td>
<td></td>
<td>150-155</td>
<td>&gt;155 or mental status changes</td>
</tr>
<tr>
<td>Low Sodium</td>
<td>130-135</td>
<td></td>
<td>129-124</td>
<td>&lt;124 or mental status changes</td>
</tr>
<tr>
<td>High Potassium</td>
<td>5.0-5.9</td>
<td>6.0-6.4</td>
<td>6.5-7.0</td>
<td>&gt;7.0 or Cardiac arrhythmias</td>
</tr>
<tr>
<td>Low Potassium</td>
<td>3.0-3.5</td>
<td>2.5-2.9</td>
<td>2.0-2.4</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>High Calcium</td>
<td>10.5-11.2</td>
<td>11.3-11.9</td>
<td>12.0-12.9</td>
<td>&gt;=13.0</td>
</tr>
<tr>
<td>Low Calcium</td>
<td>7.8-8.4</td>
<td>7.0-7.7</td>
<td>6.0-6.9</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>Low Magnesium</td>
<td>1.2-1.4</td>
<td>0.9-1.1</td>
<td>0.6-0.8</td>
<td>&lt;0.6 or Cardiac arrhythmias</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>55-65</td>
<td>40-54</td>
<td>30-39</td>
<td>&lt;30 or Mental status changes</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>116-159</td>
<td>160-249</td>
<td>250-400</td>
<td>&gt;400 or Ketoacidosis</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Tr-1+&lt;150 mg/day</td>
<td>2+150-499 mg/day</td>
<td>3+500-1000 mg/day</td>
<td>4+, or nephrotic syndrome &gt;1000 mg/day</td>
</tr>
<tr>
<td>Hematuria</td>
<td>Microscopic &lt;25 cells/hpf</td>
<td>Microscopic &gt;=25 cells/hpf</td>
<td>Gross</td>
<td>Obstruction or Transfusion requirement</td>
</tr>
</tbody>
</table>

Comments
Calcium values are corrected for albumin concentration. CrCl values do not apply to infants <2 months old.

<table>
<thead>
<tr>
<th>OTHER</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy</td>
<td>Pruritc Rash</td>
<td>Mild Urticaria</td>
<td>Severe Urticaria Anaphylaxis, Angioedema</td>
<td></td>
</tr>
<tr>
<td>Drug Fever (Rectal)</td>
<td>38.5-40</td>
<td>&gt;40</td>
<td>Sustained Fever: &gt;40, &gt;5 days</td>
<td></td>
</tr>
<tr>
<td>Cutaneous</td>
<td>Diffuse maculo-papular rash, dry desquamation</td>
<td>Vesiculation, ulcers</td>
<td>Exfoliative dermatitis, Stevens-Johnson or Erythema multiforme, Moist desquamation</td>
<td></td>
</tr>
<tr>
<td>Stomatitis</td>
<td>Mild discomfort</td>
<td>Painful, difficulty swallowing, but able to eat and drink</td>
<td>Painful: unable to swallow solids</td>
<td>Painful: requires IV fluids</td>
</tr>
<tr>
<td>SYMPTOM</td>
<td>GRADE 1</td>
<td>GRADE 2</td>
<td>GRADE 3</td>
<td>GRADE 4</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------</td>
<td>----------------------------------------------</td>
<td>----------------------------------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td><strong>CENTRAL NERVOUS SYSTEM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Seizures</em></td>
<td>None</td>
<td>Uncomplicated Sr +/- Temp Elevation</td>
<td>1 Sez/Month for &lt;=2 Consecutive Months or 3 Sez over 6 Months; No Temp Elevation</td>
<td>1 Sez/Month; No Temp Elevation; No Decrease in Sr Frequency Despite dose reduction</td>
</tr>
<tr>
<td>Seizures are a ubiquitous symptom of numerous systemic or CNS disturbances; alternative explanations should be vigorously sought and eliminated. Status epilepticus represents a severe end of the seizure spectrum, but should be considered as a single seizure event. The need for chronic or acute anticonvulsant medication should be made on a clinical basis. Seizures as a manifestation of drug toxicity are usually primarily generalized. Focal (partial onset) seizures are suggestive of focal central nervous system pathology and should be appropriately investigated, although they may be a manifestation of drug toxicity. Beware of focal seizures which secondarily generalize; these should be approached diagnostically as partial onset seizures. Children with underlying epileptic conditions who experience persistent breakthrough seizures despite maximal anticonvulsant therapy coincident with beginning the trial medication should be considered Grade 4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td>&lt;=1/Month; &lt;2 Hrs duration Mild</td>
<td>&gt;1/Month; &gt;2 Hrs Duration Moderate to Severe Responds to non-narcotic analgesia or prophylaxis</td>
<td>&gt;2/Month; &gt;2 Hrs Duration Moderate to Severe Responds to narcotic analgesia, or does not respond to prophylaxis</td>
<td>&gt;4/Month; &gt;2 Hrs Duration; Moderate to Severe; Non-Responsive to narcotic Analgesia; or persistently Recurrent despite prophylaxis No decrease in frequency or Severity despite dose reduction</td>
</tr>
<tr>
<td>Headache is a non-specific symptom, but may be a symptom of CNS/intracranial pathology. Appropriate diagnostic measures should be pursued. Duration refers to the waxing and peak phases, not to the resolution/waning phases of the headache. Mild refers to a grade of headache pain which does not affect function or activity. Moderate to severe refers to a grade of headache which affects function or activity.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mental Status And Behavior</strong></td>
<td>Changes which do not Affect Function</td>
<td>Changes requiring pharmalogic or other therapy; or mild lethargy, sedation or somnolence which resolves with rest</td>
<td>Changes not improved by drugs or other therapies; or onset of confusion, memory impairment, lethargy, sedation, or somnolence which does not respond to rest</td>
<td>Onset of delirium, obtundation, coma, or psychosis, or Grade 3 toxicity which does not respond to dose reduction</td>
</tr>
<tr>
<td>Behavior refers to the development of attention deficits with or without hyperactivity, depression, mania, agitation, sleep disorders, phobias, obsessive-compulsive behaviors, or anxiety. Mental status refers to the level of consciousness, memory function, language and analytical operations, and non-dominant hemisphere functioning. Alternative explanations should be sought.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Balance &amp; Posture</strong></td>
<td>None</td>
<td>None</td>
<td>Ataxia, dizziness, vertigo, tremor, impaired postural balance</td>
<td>Onset of movement disorder; or Grade 3 toxicity which does not respond to dosage adjustment</td>
</tr>
<tr>
<td>&quot;Ataxia&quot; can be mistakenly diagnosed in the face of central weakness or peripheral neuropathy, which should not be considered a drug toxicity of this category. Movement disorders refer to tardive or other dyskinesias, dystonias, chorea, or ballismus. Alternative explanations should be sought.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NOTE: For seizures, DO NOT GRADE BASED ON THE CRITERIA LISTED HERE. USE THE GRADING CRITERIA LISTED IN APPENDIX II-C INSTEAD*
## APPENDIX IV-B (Cont.)

<table>
<thead>
<tr>
<th>SYMPTOM</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>None</td>
<td>Blurriness, diplopia, or horizontal nystagmus of $&lt; \ 1$ hour duration, with spontaneous resolution</td>
<td>$\geq 1$ episode of Grade 2 symptoms per week, or an episode of Grade 2 $\geq 1$ episode of Grade 2 symptoms per week, or an episode of Grade 2 Symptoms lasting 1 hour with spontaneous resolution by 4 hours or vertical nystagmus</td>
<td>Decrease in visual acuity, visual field deficit, or oculogyric crisis, or Grade 3 $\geq 1$ episode of Grade 2 Symptoms per week, or an episode of Grade 3 Symptoms lasting 1 hour with spontaneous resolution by 4 hours or vertical nystagmus</td>
</tr>
</tbody>
</table>

Many of the symptoms in this category can be the result of CNS pathology, or alternatively can be an external (i.e., non-CNS) neuro-ophthalmologic disorder. Appropriate diagnostic investigations should be pursued.

### Myelopathy

| | None | None | None | Myelopathic/spinal cord symptoms, such as: Pyramidal tract weakness and disinhibition, sensory level, loss of proprioception, bladder/bowel dysfunction |

HIV can cause spinal cord syndromes rarely in children. Other infectious agents can cause myelopathies as well. Alternative explanations should be sought.

## PERIPHERAL NERVOUS SYSTEM

### Neuropathy/ Lower Motor Neuronopathy

| | None | Mild transient Paresthesia only | Persistent or progressive paresthesias, burning sensation in feet, or mild dyesthesia; no weakness; mild to moderate deep tendon reflex changes; no sensory loss | Onset of significant weakness, decrease or loss of DTRs, sensory loss in "stocking glove" distribution, radicular sensory loss, multiple cranial nerve involvement; bladder or bowel dysfunction, fasciculations, respiratory embarrassment from chest wall weakness. Grade 3 symptoms which do not resolve with dose reduction |

Infectious agents other than HIV can precipitate a neuropathy and should be considered, especially CMV. Neuropathies which do not resolve after dose reduction or discontinuation should be pursued for alternative infectious or non-infectious etiologies, since drug-related neuropathies will usually resolve after dose reduction or drug discontinuation. It should be borne in mind that many subjects will worsen for up to one month after drug discontinuation prior to improvement ("coasting"). Abnormalities should be confirmed by nerve conduction studies (NCS) +/- electromyographic studies (EMG).

### Myopathy or Neuromuscular Junction Impairment

| | Normal or mild ($< 2 \times N$) CPK elevation | Mild proximal weakness and/or atrophy not affecting gross motor function. Mild myalgias, $\sim$ mild CPK elevation ($< 2 \times N$) | Proximal muscle weakness and/or atrophy affecting motor function $\sim$ CPK elevation; or severe myalgias with CPK $> 2 \times N$; Consider confirmatory EMG and/or muscle bx | Onset of myasthenia-like symptoms (fatiguable weakness with external, variable ophthalmoplegia (and/or ptosis), or neuromuscular junction blockade (acute paralysis) symptoms (confirm with EMG); or Grade 3 symptoms which do not resolve on dose adjustment; confirm with muscle bx |

HIV can produce a myopathy, and should be differentiated. Drug-induced myopathy can be accompanied by normal CPK levels. On occasion, neuropathic or central weakness can mimic myopathic weakness.
<table>
<thead>
<tr>
<th>SYMPTOM</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical symptoms <em>not otherwise specified</em> in this table</td>
<td>No therapy; monitor condition</td>
<td>May require minimal intervention and monitoring</td>
<td>Requires medical care and possible hospitalization</td>
<td>Requires active medical intervention, hospitalization, or hospice care</td>
</tr>
<tr>
<td>Laboratory values <em>not otherwise specified</em> in this table</td>
<td>Abnormal, but requiring no immediate intervention; follow</td>
<td>Sufficiently abnormal to require evaluation as to causality and perhaps mild therapeutic intervention, but not of sufficient severity to warrant immediate changes in study drug</td>
<td>Sufficiently severe to require evaluation and treatment, including at least temporary suspension of study drug</td>
<td>Life-threatening severity. Requires immediate evaluation, treatment, and usually hospitalization. Study drug must be stopped immediately and should not be restarted until the abnormality is clearly felt to be caused by some other mechanism that study drug.</td>
</tr>
</tbody>
</table>
APPENDIX V

SUPPLEMENTAL TOXICITY TABLE FOR GRADING THE SEVERITY OF PERIPHERAL NEUROPATHY

NOTE: Use this Appendix to assess toxicity grading of peripheral neuropathy described in Section 6.4 and Appendix VI.

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

EVALUATION OF PERIPHERAL NEUROPATHY

TRUNCATED DENVER DEVELOPMENTAL TEST
AND TESTING FOR DEEP TENDON REFLEXES

Evaluation is to be performed at every study visit beginning with study entry through week 96.

Study participants should be able to pass age-appropriate (adjusted for prematurity) evaluations listed below. Performance will be rated as “yes, no, or unable to assess (subject not cooperative).”

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


APPENDIX VI (Cont.)

<table>
<thead>
<tr>
<th>AGE</th>
<th>EVALUATION</th>
</tr>
</thead>
</table>
| 15 months | Tests for peripheral neuropathy in upper extremities:  
|  | • Scribbles  
|  | • Dump bead  
|  | Test for peripheral neuropathy in lower extremities:  
|  | • Walks well |
| 18 months | Tests for peripheral neuropathy in upper extremities:  
|  | • Builds tower of 2 blocks  
|  | • Dumps bead  
|  | Test for peripheral neuropathy in lower extremities:  
|  | • Runs |
| 24 months | Tests for peripheral neuropathy in upper extremities:  
|  | • Builds tower of 4 cubes  
|  | • Throws ball overhead  
|  | Test for peripheral neuropathy in lower extremities:  
|  | • Kicks ball  
|  | • Walks up steps |

Reflexes (performance will be rated as “normal, hyperactive, hypoactive, absent, unable to assess (subject not cooperative)”):  
For upper extremities:  
• Biceps reflexes  

For lower extremities:  
• Ankle reflexes  
• Knee reflexes
# APPENDIX VII

**VIROLOGY, HEMATOLOGY AND IMMUNOLOGY COLLECTION AND SHIPPING INSTRUCTIONS**

## WHOLE BLOOD PELLETS FOR HIV DNA PCR AND STORAGE FOR REPEAT ASSAYS

<table>
<thead>
<tr>
<th>ASSAY REQUIREMENT</th>
<th>SPECIMEN COLLECTION</th>
<th>COLLECTION CONTAINER</th>
<th>IMMEDIATE SPECIMEN HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells for HIV DNA PCR - blood shared with Whole blood for Hematology</td>
<td>2.0 ml blood collected by venipuncture</td>
<td>Collect in 5 ml Tripotassium EDTA Vacutainer™ (Purple-top tube)</td>
<td>• Gently invert tubes several times to mix. Do not shake.</td>
</tr>
<tr>
<td>Plasma for HIV RNA PCR and/or HIV-1 EIA - blood shared with Whole blood for Hematology and Lymphocyte Subsets</td>
<td>3.5 ml blood collected by Venipuncture</td>
<td>Collect in 5ml Tripotassium EDTA Vacutainer™ (Purple-top tube)</td>
<td>• Specimen should be identified as to patient ID#, study ID#, site ID#, visit ID#, date and time of collection, and specimen type.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Specimen should be kept at room temperature (18-24 °C) and processed within 30 hours of collection (preferably within 4-6 hours). Specimens can be shipped overnight at room temperature.</td>
</tr>
</tbody>
</table>
APPENDIX VII (Cont.)

SPECIMEN PROCESSING

WHEN BLOOD IS TO BE SHARED FOR HIV DNA PCR AND HEMATOLOGY, 2.0 ML SPECIMEN SHOULD BE DRAWN.

1. Remove six 100 microliter aliquots of whole blood and place in vials containing 1.0 mL of Specimen Wash Buffer. Prepare Whole Blood Pellets as described below.
2. Send the remaining blood (minimum of 1.0 mL) for hematology as indicated in Appendix IA.

WHOLE BLOOD CELL PELLETS FOR HIV DNA PCR:

1. For each specimen prepare six vials (2.0 mL Sarsted vials) with 1.0 mL of specimen wash buffer (from Roche Whole Blood Specimen Kit). Note each patient sample will be run in duplicate. The processing lab should prepare a minimum of 6-tubes for each patient so that two can be sent for testing and four additional pellets can be saved for possible repeat testing.
2. Add 100 µL of whole blood to each vial containing specimen wash buffer.
3. Seal vial and mix by inversion 10-15 times.
4. Allow mixture to stand at room temperature for 5 min. Vortex specimen vial thoroughly for a minimum of 15-30 sec. and incubate at room temperature for 5 min. Vortex specimen thoroughly for a minimum of 15-30 sec.
5. Centrifuge 3 min at 1200 rpm (room temp) in a table top microfuge.
6. Aspirate supernatant being careful to avoid disturbing the pellet. Add 1.0 mL specimen wash buffer and vortex thoroughly for 30 sec. Again centrifuge for 3 min at 1200 rpm at room temp.
7. Repeat step 6.
8. Aspirate supernatant being careful to avoid disturbing the pellet. The dry pellet may be extracted immediately or stored at –70°C until ready to extract.
9. Send two pellets for testing as soon as possible.
10. Store remaining four dry cell pellets at –70°C or below for possible repeat testing.
11. All specimens should be logged onto the Laboratory Data Management System (LDMS) by the processing laboratory. Computer labels should be generated that have the LDMS specimen number, patient identification (PID), protocol number (P1041), visit identification number (VID), specimen date, specimen time, primary, derivative, additive, and sub-additive/derivative. LDMS codes: BLD/EDT/CET

BLOOD WILL BE SHARED FROM THE SAME 3.5 ML SAMPLE FOR HIV-1 RNA, HIV-1 EIA, HEMATOLOGY AND LYMPHOCYTE SUBSETS:

1. Remove 2 mL of whole blood from the vacutainer and transfer to a centrifuge tube for plasma isolation
2. Send the remaining blood (minimum of 1.2 mL) for standard hematology and lymphocyte subset assays as indicated in Appendix IA.
**PLASMA ISOLATION FOR HIV-1 RNA AND/OR EIA DETERMINATION:**

1. Centrifuge blood at 800 x g for 10 minutes at 18-24°C.
2. Transfer plasma to clean centrifuge tube; re-centrifuge at 800 x g for 10 minutes to completely remove platelets and cell debris.
3. Aliquot plasma in 0.5 ml aliquots (minimum of 2 aliquots) into sterile, labeled cryovials (label with same information as blood tubes).
4. Send one aliquot for testing as soon as possible. Store remaining aliquots at –70°C or below.
5. **All specimens should be logged onto the Laboratory Data Management System (LDMS) by the processing laboratory. Computer labels should be generated that have the LDMS specimen number, patient identification (PID), protocol number (P1041), visit identification number (VID), specimen date, specimen time, primary, derivative, additive, and sub-additive derivative. LDMS codes: BLD/EDT/PLA**

**SHIPPING:**

1. Result from HIV DNA PCR from pre-entry sample must be available to complete randomization. For subjects with positive HIV DNA PCR result at pre-entry, HIV-1 RNA results from entry and 3 month visits (12 week visit) must be available to determine whether repeat HIV DNA PCR specimen is required at 6 months (24 week visit). If only one of the two HIV-1 RNA results is available, a detectable HIV-1 RNA result would make it unnecessary to repeat the HIV DNA PCR determination and a negative result would require that the HIV DNA PCR determination be repeated at 24 weeks.
2. Unused cell pellets and plasma aliquots should be stored on site until shipping instructions are received.

**SHIPPING INSTRUCTIONS:**

**For Johannesburg:**
Contract Lab Services (CLS) University of Witwatersrand
Johannesburg Hospital, Area 454, Room 29, Jubilee Street, Parktown Johannesburg South Africa
CLS, Postnet Suite 181, Killamey 2193 South Africa
Laboratory Director for CLS – Dr. Jessica Trusler
LDMS Lab #350

Overall Director: Dr Wendy Stevens
Phone: 27-11-489-8505
Email: wendy@dlatech.co.za

**For Cape Town:**
PathCare Clinical Trials
601 Fountain Medical Centre
Adderley St
Cape Town, South Africa.
LDMS Lab #279
APPENDIX VIII

PHARMACOLOGY COLLECTION AND SHIPPING INSTRUCTIONS

<table>
<thead>
<tr>
<th>ASSAY REQUIREMENT</th>
<th>SPECIMEN</th>
</tr>
</thead>
</table>
| **Plasma (HPLC) & PBMCs (dry cell pellets)** | First Population PK (drawn at study week 0 or 12): 2ml of venous blood collected by venipuncture at 1 and 3 hours (Group 1A and 2A) or 2 and 4 hours (Group 1B and 2B) post observed INH dose*  
Second population PK and cells for genotype (drawn at study week 84): 3ml of venous blood collected by venipuncture at 1 and 3 hours (Group 1A and 2A) or 2 and 4 hours (Group 1B and 2B) post observed INH dose* |

<table>
<thead>
<tr>
<th>COLLECTION CONTAINER</th>
<th>IMMEDIATE SPECIMEN HANDLING</th>
</tr>
</thead>
</table>
| Pre-cooled EDTA tube (Purple top) | • Specimen should be identified as to patient ID#, study ID#, site ID#, visit ID#, and date and time of collection. Also include subject’s age, sex, and weight.  
• Specimen should be stored on ice and protected from light until separation of cellular components from plasma. |

SPECIMEN PROCESSING (Separation of cellular components and plasma from whole blood):

1. Separate cellular components from plasma by centrifuge (While centrifuging, the temperature should be no greater than 10°C. Centrifuge at 3500 gravitation for 5 minutes) Caution: Be careful not to cause hemolysis. Eppendorf tubes can be used.
2. Remove supernatant (plasma) and place a minimum of 1.0 - 1.5 ml into 2 separate Eppendorf tubes. Store plasma samples on dried ice (-80°C) and transport to the designated pharmacology lab in shielded container within 24 hours of collection. Each sample should be handled and marked separately.
3. Save the pellets of each of sampling period and combine them into one EDTA vacutainer (Purple top) (2 - 3 ml)
4. Label and store the pellet at ≤ 80°C for future NAT-2 genotyping.
5. **All specimens should be logged onto the Laboratory Data Management System (LDMS) by the processing laboratory.**
Computer labels should be generated that have the LDMS specimen number, patient identification (PID), protocol number (P1041), visit identification number (VID), specimen date, specimen time, primary, derivative, additive, and sub-additive/derivative. LDMS codes: BLD/EDT/PLA (PK) and BLD/EDT/CEL (NAT-2 genotyping)

*Draw as close to these times as possible. Write down exact times these blood samples were drawn.

<table>
<thead>
<tr>
<th>DESIGNATED LABORATORY/CONTACT PERSONS:</th>
<th>NAT-2 GENOTYPING:</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHARMACOLOGY / INH CONCENTRATION:</td>
<td>Professor Paul v Helden</td>
</tr>
<tr>
<td></td>
<td>Director; MRC Centre for Molecular and Cellular Biology</td>
</tr>
<tr>
<td></td>
<td>Head, Dept. Medical Biochemistry</td>
</tr>
<tr>
<td></td>
<td>ATTN: Cedric Werely</td>
</tr>
<tr>
<td></td>
<td>Room F412A (Secretary)</td>
</tr>
<tr>
<td></td>
<td>Faculty of Health Sciences</td>
</tr>
<tr>
<td></td>
<td>Stellenbosch University</td>
</tr>
<tr>
<td></td>
<td>Francie van Zijl Drive</td>
</tr>
<tr>
<td></td>
<td>Tygerberg, South Africa</td>
</tr>
<tr>
<td></td>
<td>Phone: +27 (0) 21 9389401 or 9124</td>
</tr>
<tr>
<td></td>
<td>Fax: +27 (0) 21 9317841 or 9389476</td>
</tr>
<tr>
<td></td>
<td>E-mail: <a href="mailto:pvh@sun.ac.za">pvh@sun.ac.za</a></td>
</tr>
</tbody>
</table>

SHIPPING (e.g., real-time vs. batched; required forms):

<table>
<thead>
<tr>
<th>PLASMA:</th>
<th>PBMCs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ship within 24 hrs of collection.</td>
<td>Keep frozen at ≤ 80° C in an EDTA tube (purple top). Ensure PBMCs are appropriately labeled with at least: patient ID#, study ID#, site ID#, visit ID#, Can be batch shipped or shipped with respective plasma sample on dry ice/at 80°C.</td>
</tr>
</tbody>
</table>

| INCLUDE the pkw1 form | |

**OTHER INSTRUCTIONS:** Please contact Dr. Seifart, by email or fax, 24 hours prior to shipping specimens.
APPENDIX IX

METHODS FOR NASOPHARYNGEAL SWABS FOR THE DETECTION OF
STREPTOCOCCUS PNEUMONIAE

NOTE: THIS PROCEDURE WILL ONLY BE DONE AT JOHANNESBURG.
All specimens should be logged onto the Laboratory Data Management System (LDMS) by the processing laboratory. Computer labels should be generated that have the LDMS specimen number, patient identification (PID), protocol number (P1041), visit identification number (VID), specimen date, specimen time, primary, derivative, additive, and sub-additive/derivative.
LDMS codes: NPM/NON/SWB/STG

I – PROCEDURE FOR OBTAINING NASOPHARYNGEAL SWABS TO DETERMINE PNEUMOCOCCAL COLONIZATION

1. Order STGG (skim milk-tryptone-glucose-glycerin) medium in Nunc cryotubes (1 ml aliquots) from Diagnostic Media Products (DMP), NHLS, and store in a fridge at 4°C.

2. Quality control for each new batch must include sterility check and growth performance (this needs to be done by RMPRU staff and documented for each batch).

3. Shelf-life of STGG: 6 months if stored at 4°C.

4. Order paediatric calcium alginate swabs with flexible aluminium shaft (recent suppliers are MW&E, Medical Wire & Equipment Co. Ltd., Corsham, Wiltshire, England) and store at room temperature.

5. Type of swab may not be changed during a trial at a specific site.

6. Prior to use: resuspend the pellet/precipitate by vortexing for 10 to 20 seconds. This can be done in the laboratory before going to the wards.

7. The child’s/adult’s head is tipped slightly backward, with the individual taking the specimen placing his/her hand behind the patient’s neck (if a child the mother/guardian can place the child on his/her lap and hold the child around the waist and on his/her forehead).

8. The above-mentioned swab is passed directly backwards through the nostril of the child/adult without tipping the swab up or down (the nasal passage runs parallel to the floor and not parallel to the bridge of the nose).
9. Do not use force while inserting the swab, rotating the swab may help.

10. If resistance is noted, remove the swab and try the other nostril.

11. Once the swab is in place (a distance that is almost half the distance between the nostril and the lobe of the ear), rotate the swab 180° and leave it in place for 5 seconds to saturate the tip.

12. Remove slowly.

13. Record the presence of snot.

14. Record whether the procedure was suboptimal.

15. Label transport medium container (Nunc cryotube from DMP) containing 1ml of well mixed, vortexed STGG with study number and specimen number.

16. Label the specimen log sheet and record other data as required.

17. Place the swab into the labelled transport vial.

18. Using scissors cleaned with an alcohol wipe, aseptically cut off the excess wire handle from the swab and close the cap.

II – TRANSPORT AND STORAGE OF THE SPECIMEN

1. Place the specimen in a plastic bag, place bag on wet ice and transport to the Respiratory and Meningeal Pathogens Research Unit, National Institute for Communicable Diseases, 1 Modderfontein, Sandringham, South Africa within 8 hours.

2. As soon as received in the laboratory, vortex the specimen for 10 to 20 seconds to disperse organisms from the calcium alginate swab.

3. Freeze immediately at -70°C in the STGG transport medium (the swab is left in the medium).

III – PROCESSING OF STGG FOR THE GROWTH OF STREPTOCOCCUS PNEUMONIAE

1. Order 5% horse blood, Columbia based, agar plus 5mg/ml of gentamicin sulfate from Diagnostic Media Products (DMP), NHLS, and store in a fridge at 4°C.
2. Quality control of each batch is performed at DMP.
APPENDIX IX (Cont.)

3. Ensure all procedures for incubation at 5% CO₂ at 37°C are in place.

4. Remove relevant STGG vials from the -70°C freezer.

5. Thaw by leaving at room temperature for several minutes (up to 30 minutes) until the medium becomes liquid.

6. Vortex vial of STGG for 10 to 20 seconds.

7. Label selective medium agar plates (5% horse blood plus gentamicin).

8. Inoculate a predetermined volume (using a bacteriological loop calibrated to deliver a volume of approximately 0.001ml) of the STGG medium onto selective medium agar plates. Use one plate per specimen.

9. Streak or thin out for single colonies with a sterile bacteriological loop.

10. Incubate agar plates at 5% CO₂ at 37°C overnight (18 to 24 hours).

11. Reincubation for a total of 48 hours may be required if no suspicious colonies are seen after 24 hours.

12. Label non-selective agar plates (5% horse blood) as required.

13. Pick off and streak out on one half of a blood agar plate (without gentamcin) two presumptive pneumococcal colonies (alpha haemolysis on blood agar, macroscopic morphology may be mucoid, transparent or draughtsman-like), looking as different as possible.

14. Place an optochin disc in the centre of both inoculations.

15. Incubate agar plates at 5% CO₂ at 37°C overnight.

16. Confirm colonies are *Streptococcus pneumoniae*: susceptible to optochin (ethyl hydrocupreine hydrochloride, 5µg; Becton Dickinson Microbiology Systems, Cockeysville, MD) i.e. zone ≥ 14mm; if zone is smaller, bile solubility is occasionally performed as an alternative identification test.
APPENDIX IX (Cont.)

17. *S. pneumoniae* colonies are re-subcultured for heavy growth as a lawn for harvesting with a sterile swab for dispensing into 1 tube (carefully labeled) with 10% skim milk and then placed at -70°C storage.

18. If different morphotypes are identified, store each in a separate tube.

19. Serotyping and susceptibility are tested as described below.

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>No colonies suspicious of <em>pneumococci</em></td>
</tr>
<tr>
<td>Scanty</td>
<td>&lt; 25 colonies in quadrant 1</td>
</tr>
<tr>
<td>1+</td>
<td>≥ 25 colonies in quadrant 1 plus &lt; 25 colonies in quadrant 2</td>
</tr>
<tr>
<td>2+</td>
<td>≥ 25 colonies in quadrant 2 plus &lt; 25 colonies in quadrant 3</td>
</tr>
<tr>
<td>3+</td>
<td>≥ 25 colonies in quadrant 3 plus &lt; 25 colonies in quadrant 4</td>
</tr>
<tr>
<td>4+</td>
<td>≥ 25 colonies in quadrant 4</td>
</tr>
</tbody>
</table>

III – SUSCEPTIBILITY TESTING

Test all isolates for susceptibility to the following antibiotics by disc diffusion: oxacillin 1 µg, chloramphenicol 30 µg, tetracycline 30 µg, erythromycin 15 µg, clindamycin 2 µg, rifampicin 5 µg, cotrimoxazole 25 µg, levofloxacin 5 µg (MASTRING-S, Mast Diagnostics, Mast Group Ltd., Bootle, Merseyside, UK).

NOTES:
- This susceptibility testing is in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.
- All isolates resistant to any of the above antibiotics by disc diffusion screening will have Minimum Inhibitory Concentration (MIC) testing.
- Penicillin and ceftriaxone MICs are done by the agar dilution methodology according to the NCCLS guidelines.
- All other MICs are done by the E-test methodology according to the NCCLS guidelines.

IV – SEROTYPING OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES

1. Serotyping will be performed using the Quelling reaction.
2. Subculture isolates stored in -70°C (skim milk in cryotubes) onto 5% horse blood agar and incubate at 37°C in 5% CO₂ for 24 hrs.

APPENDIX IX (Cont.)

Note: Subculturing from this overnight culture and repeat incubation as above is performed if typing described below is unsuccessful.

3. A saline suspension of 0.5ml is made of overnight growth of the isolate to an opacity corresponding to less than McFarland standard 0.5.

4. Label glass slide.

5. Approximately one loopful (0.001 ml) of the culture suspension above is placed to make two smears on a clean glass slide.

6. Several smears are made in the same way. The slides are allowed to air dry.

7. Antisera (obtained from the Statens Seruminstitut, Copenhagen, Denmark) in 10 µl aliquots from groups A to I are added to and mixed well with 10 µl of methylene blue on cover slips.

8. These cover slips are inverted and placed over the smears on the slides.

9. The slides with cover slips are allowed to stand for 10 to 15 minutes and then examined under ×1000 magnification using an oil-immersion objective.

10. The same procedure is repeated for the serogroup- or serotype-specific antisera.
APPENDIX X

NAT2 GENOTYPING PROTOCOL

DNA Extraction

DNA will be purified from blood samples (collected in EDTA tubes) using a salting out procedure [Miller et al., 1988]. Essentially this entails rupturing the cell wall (cell lysis, with subsequent removal of the cellular debris by centrifugation) followed by selective digestion of the nuclear protein (with Proteinase K, 37°C, overnight). The addition of a high salt solution (6M NaCl) enables the precipitation of the protein - removed by centrifugation – and subsequent precipitation of the DNA with absolute ethanol.

PCR Amplification

A 1000 base pair NAT2 sequence is amplified by the polymerase chain reaction (PCR) as previously described [Hickman and Sim, 1991]. This 1000bp segment is then analyzed using the restriction fragment length polymorphism (RFLP) technique, with appropriate cleavage by the restriction enzymes BamHI, KpnI, Mpl, and TaqI; these enzymes delineate the 2*7, 2*5, 2*14 and 2*6 alleles respectively. Additional analyses of the NAT2-PCR product with the restriction enzymes Ddel and FokI, as well as the inclusion of an allele-specific PCR reaction (for the T341C mutation) allow for the sub-classification of these NAT2 alleles.

Gel Electrophoresis

The NAT2 DNA cleavage profiles, generated by each of the restriction enzymes are analysed by gel electrophoresis using MetaPhor® agarose (Biowhittaker, USA), and the profiles are visualized under ethidium bromide staining.

References

APPENDIX XI

INSTRUCTIONS FOR CRUSHING INH/PLACEBO TABLETS FOR ADMINISTRATION

Whenever possible, warm water should be used when crushing INH/Placebo tablets for administration.

The correct number of tablet pieces for your child is _____ whole tablets and _____ half tablets.

1. Obtain the correct number of tablets and half tablets.

2. Break the tablet(s) into pieces no larger than ¼ of a tablet.

3. Use identical metal spoons (or mortar and pestle) to crush the pieces.

4. Continue crushing until a fine powder results.

5. Transfer the powder to a small glass or cup or a large bowl spoon. (You need to be able to reach the bottom of the container with the tip of an oral syringe.)

6. Add 5-6 drops of warm water to wet the powder and work into a paste.

7. Add approximately ½ teaspoon (2.5 ml) of warm water and work into a milky solution.

8. Withdraw all that you can with an oral syringe.

9. Rinse the cup/glass/spoon with another 1-2 ml of warm water to get the residual drug into solution.

10. Withdraw the additional drug with the rinse water into an oral syringe.

11. Administer both syringes to the child inserting the syringe into the side of the mouth to avoid the taste buds on the tongue.
**APPENDIX XII**

**Enrollment Log for P1041**

*Site Instructions*: Please enter data for all children identified to be approached for P1041 at your site, with whom you made contact during the preceding quarter (whether or not enrolled in P1041). The race/ethnicity categories are appropriate where enrolling sites are located. Please fill in counts in all the blank fields (put zero where appropriate).

(Note: For the calendar quarter in which the site first begins randomizing participants to be approached for P1041, the log should include information on all children from the date of the first randomization through the end of the quarter.)

Log for _____ Quarter _____ (Start Date of Log: __________ - End Date: __________) *

(quarter #) (year)

Site Code: _________________

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total number of children screened in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total children approached for enrollment to P1041</td>
<td></td>
</tr>
<tr>
<td>Age of mother (years):</td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td></td>
</tr>
<tr>
<td>20 – 24</td>
<td></td>
</tr>
<tr>
<td>25 – 29</td>
<td></td>
</tr>
<tr>
<td>30 – 35</td>
<td></td>
</tr>
<tr>
<td>&gt; 35</td>
<td></td>
</tr>
<tr>
<td>unknown/missing</td>
<td></td>
</tr>
<tr>
<td>History of maternal antiretroviral use:</td>
<td></td>
</tr>
<tr>
<td>ART initiated before labor</td>
<td></td>
</tr>
<tr>
<td>ART initiated during labor</td>
<td></td>
</tr>
<tr>
<td>ART initiated after delivery</td>
<td></td>
</tr>
<tr>
<td>No ART</td>
<td></td>
</tr>
<tr>
<td>unknown/missing</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity of child:</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td></td>
</tr>
<tr>
<td>Coloured</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>unknown/missing</td>
<td></td>
</tr>
<tr>
<td>Children registered (enrolled) in P1041?:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

* Quarter 1: January 1-March 31, Quarter 2: April 1-June 30, Quarter 3: July 1-Sept 30, Quarter 4: Oct 1-Dec 31.
APPENDIX XII (Cont.)

Enrollment Log for P1041 (cont.)

For all children screened but not enrolled in P1041, indicate the PRIMARY reason for non-participation below. ALL non-enrollments need to be included in one of the following categories. Each child should be counted ONLY once.

<table>
<thead>
<tr>
<th>Reason not enrolled</th>
<th>Total number of children in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Child not eligible to participate:</td>
<td></td>
</tr>
<tr>
<td>a. Child on protocol that prohibits co-enrollment with P1041</td>
<td></td>
</tr>
<tr>
<td>b. Child cannot be followed at the site (e.g., does not receive routine care at the site)</td>
<td></td>
</tr>
<tr>
<td>c. Child too old for enrollment</td>
<td></td>
</tr>
<tr>
<td>d. BCG received vaccine after 7 days of age</td>
<td></td>
</tr>
<tr>
<td>e. Child current/previous TB or has been in contact with a TB case</td>
<td></td>
</tr>
<tr>
<td>f. Lab value outside acceptable range</td>
<td></td>
</tr>
<tr>
<td>g. Child not eligible for P1041 for other reasons</td>
<td></td>
</tr>
<tr>
<td>h. No informed consent document available in a language that the parent/guardian understands</td>
<td></td>
</tr>
<tr>
<td>2. Child/parent/guardian refused to participate due to:</td>
<td></td>
</tr>
<tr>
<td>a. Insufficient/lack of time</td>
<td></td>
</tr>
<tr>
<td>b. Travel difficulties/site too far</td>
<td></td>
</tr>
<tr>
<td>c. Co-enrollment on other protocol(s), specify:</td>
<td></td>
</tr>
<tr>
<td>d. Concerns about confidentiality</td>
<td></td>
</tr>
<tr>
<td>e. Concerns about specimen collection</td>
<td></td>
</tr>
<tr>
<td>f. Mistrust of research</td>
<td></td>
</tr>
<tr>
<td>g. Other reasons, specify:</td>
<td></td>
</tr>
<tr>
<td>3. Clinician decided not to enroll the child. NOTE: this should only be used under extraordinary circumstances, specify:</td>
<td></td>
</tr>
<tr>
<td>4. Child/parent/guardian not available/could not be reached for consent and potential enrollment in P1041</td>
<td></td>
</tr>
<tr>
<td>5. Approach/consent/registration in progress (incomplete)</td>
<td></td>
</tr>
<tr>
<td>6. Other, specify:</td>
<td></td>
</tr>
</tbody>
</table>

MAILING INSTRUCTIONS:

Complete the Enrollment Log and mail or fax to the Data Management Center:

FAX:  716-834-8675  MAIL:  Frontier Science Foundation
Attn: Barbara Nowak  4033 Maple Road
Amherst, NY 14226
USA

Attn: Barbara Nowak

The Enrollment Log may also be emailed back as an attachment to: nowak.barbara@fstrf.org
APPENDIX XIII-A

DIVISION OF AIDS

SAMPLE INFORMED CONSENT

IMPAACT P1041

A Randomized, Double Blind, Placebo Controlled Trial to Determine the Efficacy of Isoniazid (INH) in Preventing Tuberculosis Disease and Latent Tuberculosis Infection among Infants with Perinatal Exposure to HIV

Short Title: Efficacy of Isoniazid to Prevent Tuberculosis in Infants Perinatally Exposed to HIV

INTRODUCTION

Your child is being asked to take part in this research study because either you or the biological mother is infected with HIV. This study is sponsored by the U.S. National Institutes of Health (NIH). The doctor in charge of this study at this site is: insert name of Principal Investigator. Before you decide if you want your child to be a part of this study, we want you to know about the study.

This consent form gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to allow your child to take part in this study, you will be asked to sign this consent form. You will get a copy to keep. If your child does not join this study for any reason we would still like to use some of your child’s information. You are being asked to allow the study staff to collect some information about your child such as their age, sex, race, any illnesses or diagnosis, and results of blood tests. A code number that will not identify your child by name will be used to keep this information.

WHY IS THIS STUDY BEING DONE?

Children who are HIV-infected may develop serious chest infections (pneumonia) because HIV causes a child’s immune system (the body’s defense against infection) to become weaker with time. Two very serious types of pneumonia in children are caused by the tuberculosis germ which causes TB disease and the Pneumocystis carinii germ which causes pneumocystis jirovici (previously carinii) pneumonia (PCP). When these types of pneumonias occur in children who are younger than two years of age and who are infected with HIV, hospitalization or death can result. Also, all children, even if they are not infected with HIV, are more likely to become infected with TB if someone else in their household is also infected with both HIV and TB. However, HIV-infected children who also become infected with TB may develop TB disease.
faster than children who do not have HIV infection. Some other studies have shown that INH can help HIV-infected children live longer, especially those who are very sick with HIV disease.

This study is being done to find out if children who are born to HIV-infected mothers may benefit from treatment with an antibiotic called isoniazid (INH). INH may prevent these children from becoming infected with TB or ill with TB disease if they are already infected with the TB germ. The study will also show whether INH is safe for children if it is used for two years.

Many studies have shown that most cases of PCP can be prevented in HIV-infected children if they receive regular treatment with an antibiotic called TMP/SMX. The World Health Organization recommends using TMP/SMX in infants whose mother's have HIV until the child is found to be HIV uninfected or until they turn one year old. This study will look at the number of children who get PCP.

In this study, about 1300 children will be asked to take part. Of these, 800 children will be HIV-uninfected and 500 children will be HIV-infected. Half of the HIV-infected children and half of the HIV-uninfected children will receive INH and the other half will receive placebo (a tablet that looks like the INH tablet, but that contains no medicine). Your child's chance of getting INH or placebo is the same, just like flipping a coin. Only HIV-infected infants will be enrolled into this study now because in June 2006 the number of HIV-uninfected infants we needed in the study was reached. In order for us to enroll 500 HIV-infected children, we will need to test more children. If your child has been tested to be in the study, but the number of children we need for the HIV-infected group is already filled, your child will not continue in the study and your child's doctor will discuss with you the best available therapy for your child.

TMP/SMX will be given to all children who are HIV-infected and also to those who are not yet known to be HIV-infected. If your child is HIV-infected, your child will continue getting TMP/SMX until one year of age or longer if needed. Your child’s doctor will discuss with you if your child needs to continue taking TMP/SMX after one year of age. If your child’s HIV infection status has not been determined, your child will have a repeat HIV test at 6 months of age and TMP/SMX will be continued if your child is found to be HIV infected or if your child is found to be HIV-uninfected but still breastfeeding. If your child has a bad side effect while taking TMP/SMX, your child's doctor will give your child either dapsone or atovaquone, two other medicines also used to treat PCP.

WHAT DOES MY CHILD HAVE TO DO IF HE/SHE IS IN THIS STUDY?

If you agree to allow your child to participate, your child will be randomized (like the toss of a coin) to have an equal chance of getting INH or placebo (a tablet that looks like INH but does not have any real medicine in it). All children will receive INH (or placebo) once a day for 2 years and will be followed on study for two years after finishing the INH.

APPENDIX XIII-A (Cont.)
APPENDIX XIII-A (Cont.)

In addition to receiving INH or placebo, your child will also be given TMP/SMX in liquid form by mouth once a day.

At the beginning of the study, you will be asked how you would like to receive your child’s medication every month. You can choose to come to the clinic to pick it up or have a field worker bring it to your home. You should take your child to the study doctor at any time if he/she gets ill before the next scheduled study visit or if you have any concerns about your child’s health.

STUDY TESTS

For HIV-infected children:

If your child is HIV-infected, your child will have 18 study visits during the study. After the first two visits, your child will be seen every 3 months for four years. You will be informed of the results of your child’s tests during the study.

The following tests will be performed.

Pre-entry

Before your child enters the study, he/she will be checked to see if he/she is infected with HIV. About a half a teaspoon of blood will be taken from your child for this test. You will be informed of the results of your child’s test. A history and physical examination and about ½ teaspoon (3 mL) of blood will also be taken to make sure it is safe for your child to enter the study.

Entry

When your child enters the study, a history and physical examination will be done to make sure s/he is in good health, and about ¼ teaspoon (1 mL) of blood will be taken to see how well your child’s liver is working. A history and physical examination will be done at every visit (17 more times) during the study and you will be asked if your child has had any problems since his/her last visit. A developmental test to check for peripheral neuropathy (numbness and tingling in the hands and feet) will also be done by testing your child’s reflexes and playing a few games with your child. This test will be done at every study visit (8 more times) for the first two years. You will also be asked questions about how well your child is taking his/her medicines. These questions will be asked at every study visit (8 more times) for the first two years (or at least three times per year) and 2-3 more times during the next two years if your child is still taking TMP/SMX.
APPENDIX XIII-A (Cont.)

On study

At every study visit for the four years your child is in the study, about ¼ teaspoon (1mL) of blood will be drawn for a complete blood count. In addition, blood samples will be taken for other tests. At the 3, 6, 9, 12, 15, 18, 21, and 24 month visits, about ¼ teaspoon (1mL) blood will be taken to see how well your child’s liver is working. At the 9, 15, 24, 30, 36, 42, and 48 month visits, about ¼ teaspoon (1mL) of blood will be taken to see how well your child’s body is able to fight infection. At the 3, 9, 15, 24, 30, 36, 42, and 48 month visits, about ¼ teaspoon (1mL) will be taken to count the amount of HIV in your child’s blood.

For HIV-uninfected children (closed to enrollment since June 2006)

If your child is HIV-uninfected, your child will have 14 study visits during the study. After the first two visits, your child will be seen every 3 months for the first two years (at 3, 6, 9, 12, 15, 18, 21, and 24 months) to week 96, and every 6 months for the next two years (at 30, 36, 42 and 48 months) to week 192. You will be informed of the results of your child’s tests during the study.

The following tests will be performed.

Pre-entry

Before your child enters the study, he/she will be checked to see if he/she is infected with HIV. About a half a teaspoon of blood will be taken from your child for this test. You will be informed of the results of your child’s test. A history and physical examination and about 1/2 teaspoon (3 mL) of blood will also be taken to make sure it is safe for your child to enter the study.

Entry

When your child enters the study, a history and physical examination will be done to make sure s/he is in good health, and about ¼ teaspoon (1 mL) of blood will be taken to see how well your child’s liver is working. A history and physical examination will be done at every visit (12 more times) during the study and you will be asked if your child has had any problems since his/her last visit. A developmental test to check for peripheral neuropathy (numbness and tingling in the hands and feet) will also be done by testing your child’s reflexes and playing a few games with your child. This test will be done at every study visit (8 more times) for the first two years. You will also be asked some questions about how well your child is taking his/her medicines and these questions will be asked at every study visit (8 more times) for the first two years (or at least three times per year.)
On study

At the 3, 15, 21, 24, 30, 36, and 48 month visits, about ¼ teaspoon (1mL) blood will be taken to see how well your child’s liver is working. At the 6 month visit, about ¼ teaspoon (1mL) of blood will be taken to make sure that your child is not infected with HIV. At the 9 month visit, about ½ teaspoon (3mL) of blood will be taken to see how well your child’s liver is working and to make sure again that your child is not infected with HIV.

For both HIV-infected and HIV-uninfected children while on study.

The following tests will also be done while your child is on study.

**Tuberculosis (TB) Skin Test**

Your child will have a TB skin test done 3 times during the study (at the 24, 36, and 48 month visits) to see if s/he has been infected with TB. A thin needle will be placed into the skin and a substance (tuberculin) will be injected, which may cause a bump in the skin. This bump will usually disappear on its own after two to three days. If your child is infected with TB, a hard bump may appear. You will have to take your child to the clinic after 48-72 hours, so that a doctor can check the area where the tuberculin was injected.

**Nasopharyngeal Swab (For Children at Johannesburg ONLY)**

Your child will have a nasopharyngeal swab done to help us find out whether the TMP/SMX that your child is getting may have caused your child’s germs to become resistant to the TMP/SMX. If this happens, it means that the TMP/SMX may not work to stop some types of infections anymore. A thin swab will be placed through your child’s nose and passed into the back of his/her throat. This swab will then be removed and sent for testing. This test will be done 6 times during the study at the 3, 6, 9, 18, 30 and 42 month visits.

**Induced Sputum**

If your child is thought to be infected with TB and/or developed TB or PCP, an induced sputum test (making your child cough) will be done. Your child will breathe in some salt water through a mask that will make him/her cough up mucus. The mucus will then be taken out with a small tube and used to help us to look for germs that cause the chest infection.

**Nasopharyngeal and Gastric Aspirates**

If your child is thought to be infected with TB and/or developed TB or PCP, your child will have two special tests taken called nasopharyngeal aspirate and gastric aspirates (fluids). The nasopharyngeal aspirate is done by placing a small amount of salt water into your child’s nose. A small tube will then be placed through the nose and into the back of the nose to collect some fluid. The fluids that are collected from the nose will be tested for the presence of TB and PCP.
APPENDIX XIII-A (Cont.)

The gastric aspirate is done by placing a small tube through your child’s nose into the stomach to collect some fluid from the stomach. The fluids from the stomach will be tested for TB.

Storage of Blood Samples
At 5 study visits, some study blood obtained for routine testing will be stored (making sure to protect your and your child’s identity) and used for future IMPAACT approved HIV related research. A ¼ teaspoon (1mL) of blood will be drawn at each visit and will be used for this purpose. You may choose not to allow your child’s blood to be stored, but this will not prevent your child from participating in this study. You may withdraw consent for the use of your child’s specimens at any time. Once you withdraw your consent, these samples will no longer be used and will be destroyed.

These stored samples will only be used to learn more about HIV infection and its complications, and TB and will not benefit your child directly. The research may include studies to understand how HIV causes diseases and complications and its relation to TB. Testing may include studies of HIV, studies of other infections that affect people with HIV (for example hepatitis viruses), studies of your child’s cells, proteins, and other chemicals in your child’s body, and studies of your child’s genes (DNA). If any research involves the study of your child’s genes, the results will ONLY be used to find out how likely your child is to be HIV-infected, to develop AIDS or TB, and not to any other disease.

The researchers do not plan to contact you or your regular doctor with any results from these studies done on your child’s stored samples. This is because research tests are often done with experimental procedures and, in general, results from one research study should not be used to make a decision on how to treat your child. Should a rare situation come up where the researchers decide that a specific test would provide important information for your child’s health, the researchers will notify your child’s study doctor and they will try to contact you. If you wish to be contacted with this type of test result, you must give your child’s study doctor or nurse your most current contact information.

These samples will not be sold or used directly to produce commercial products. Research studies using your child’s stored samples will be reviewed by the National Institutes of Health (NIH).

These blood samples will not be stored for more than five years.

Please carefully read the statement below and think about your choice. Your decision will not affect your child’s care. Your child’s samples will be destroyed if you withdraw your consent for your child’s participation in this study.
APPENDIX XIII-A (Cont.)

I agree to have study blood (up to 1 teaspoon) taken from my child for the purpose of storage for future research related to HIV infection and its complications and TB.

_____ Yes  _________ Date _____  Initials
_____ No  _________ Date ______ Initials

HOW MANY CHILDREN WILL TAKE PART IN THIS STUDY?

About 1300 children will take part in this study; 800 HIV-uninfected children and 500 HIV-infected children.

HOW LONG WILL MY CHILD BE IN THIS STUDY?

Your child will be in this study for about four years.

WHY WOULD THE DOCTOR TAKE MY CHILD OFF THIS STUDY EARLY?

The study doctor may need to take your child off the study early without your permission if:

- the study is cancelled by the National Institutes of Health (NIH), the South African Medicine Control Council (MCC), or the in-country or the site’s Institutional Review Board (IRB) or Ethics Committee (Committees that watches over the safety and rights of research subjects.)
- a Data Safety Monitoring Board (DSMB) recommends that the study be stopped early (A DSMB is an outside group of experts who monitor the study.)
- your child is not able to attend the study visits as required by the study

The study doctor may also need to take your child off the study medication(s) without your permission if:

- continuing the study medication(s) may be harmful to your child
- your child needs a treatment that your child may not take while on the study medication(s)
- your child is not able to take the study medication(s) as required by the study
- your child needs to be treated with steroids for a long period of time
- your child is diagnosed with TB disease or TB infection
- your child is exposed to TB and is given INH to prevent TB disease

During the Study: If your child must stop taking the INH before the study is over, the study doctor will discuss other options that may be of benefit to your child, and may ask your child
to return for a study visit that may include blood tests and other procedures that will be explained to you.

**After the Study:** After your child completes this study the study will no longer give your child the INH your child got while on study. If your child still needs this medicine, the study doctor will talk to you about how to get the medicine for your child.

**WHAT ARE THE RISKS OF THE STUDY?**

Taking part in this study may involve some risks or discomforts. These include side effects of INH and TMP/SMX and possible risks/discomforts of the study tests.

1) **Side effects of INH** may include: changes in vision; clumsiness or unsteadiness; changes in the color of urine; loose or watery stools; loss of appetite, weight loss, nausea (feeling sick to the stomach) and vomiting; pain in upper abdomen; skin rash; stools lighter in color; fever; tingling and numbness in the hands and feet; weakness and fatigue; yellowing of eyes or skin. In general, these side effects are temporary. There may also be an increase in some liver function tests indicating that there may be some damage to the liver. It is possible that there may be some additional or more serious side effects that have not been seen before because INH has not been used for 2 years continually in young children.

2) **Side effects of TMP/SMX** may include skin rash; nausea (feeling sick to the stomach) and vomiting; neutropenia (decrease in neutrophils, a type of white blood cell, which helps fight infection); loss of appetite; sensitivity to light; stomach pain; anemia (decrease in the number of red blood cells that may cause dizziness or feeling tired); loose or watery stools; difficulty sleeping; weakness and fatigue, yellowing of eyes or skin. In general, these side effects are temporary.

3) **Side effects of Dapsone**

Side effects include mainly blood abnormalities: anemia (decrease in number of red blood cells that carry oxygen), neutropenia (decrease in number of white blood cells that fight infection), hemolysis (destruction of the red blood cells) and methemoglobinemia (a condition in which hemoglobin cannot carry oxygen from the lungs to the tissues). Skin rash, feeling sick to the stomach, vomiting and liver problems have been reported but are not permanent. Other rare problems include exfoliative dermatitis (skin irritation with redness and flaking), liver abnormalities and peripheral neuropathy (pain or weakness in the hands or feet).
APPENDIX XIII-A (Cont.)

4) Side Effects of Atovaquone

The more common side effects include fever and skin rash. Other side effects such as cough, diarrhea, headache, nausea, trouble sleeping, feeling sick to the stomach, or vomiting may occur. If you notice any other effects, check with your child's doctor.

5) Blood drawing may cause some pain, discomfort, bleeding, or bruising where the needle enters the skin. Blood drawing may cause a lightheaded feeling, and in rare cases fainting. A small blood clot may form at the site where the needle enters the skin or swelling of the surrounding skin may occur. There is also a small risk of a minor infection at the blood draw site.

6) Nasopharyngeal and gastric aspirates and induced sputum. The risks of obtaining samples of fluid from the back of your child's throat and stomach are that these procedures may cause your child to cough for a little while, but the coughing should not last long. In addition, your child may feel some discomfort or experience minor bleeding when the tube is placed in his/her nose. If bleeding does occur, it will stop on its own.

7) Nasopharyngeal swab (For Children at Johannesburg ONLY)
The nasal swab taken from the back of your child’s nose to look for germs may cause coughing, minor discomfort and some bleeding.

8) Other risks. There may be other risks to taking part in this study that are not known at this time. For your child’s safety, you must tell your child’s doctor or nurse about all medications your child is taking/getting before starting the study, and also before starting any new medications while on study; including over-the-counter medications and herbal or natural remedies. In addition, you must tell the study doctor or nurse before your child enrolls in any other clinical trials while on this study.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If your child takes part in this study, there may be a direct benefit to your child, but no guarantee can be made. It is also possible that your child may receive no benefit from being in this study. Information learned from this study may help others who are HIV-infected. It may also help those in families affected by HIV and who have or might become infected TB and PCP.

Another possible benefit of taking part in this study is that your child will be tested to find out whether or not your child in HIV-infected at 4 months of age rather than waiting until s/he is older. The test that we will use is not freely available at government clinics and/or hospitals for children. An earlier diagnosis of HIV infection may be helpful.
APPENDIX XIII-A (Cont.)

WHAT OTHER CHOICES DOES MY CHILD HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- treatment with prescription drugs available to your child
- treatment with other study drugs, if your child qualifies to take part in available studies
- no treatment

Please talk to your doctor about these and other choices available to your child. Your doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your and your child’s personal information confidential. We cannot guarantee absolute confidentiality. Your and your child’s personal information may be disclosed if required by law. Any publication of this study will not use your/your child’s name to identify you/your child personally.

Your/your child’s medical records may be reviewed by the (insert name of site) IRB/EC, National Institutes of Health (NIH), Secure the Future Fund (a group that is paying for some of the costs of the study), study staff, or study monitors.

WHAT ARE THE COSTS TO ME AND MY CHILD?

You will not be expected to pay for the study medicines, INH and TMP/SMX, the study related visit or study procedures. If your child cannot take TMP/SMX and needs dapsone or atovaquone, these medicines will not be supplied by the study.

WILL I OR MY CHILD RECEIVE ANY PAYMENT?

Participation in this study is voluntary. You will be reimbursed for all transportation and meal expenses due to participation in this study.

WHAT HAPPENS IF MY CHILD IS INJURED?

If your child is injured as a result of being in this study, (name of research unit) will give your child immediate necessary treatment for the injuries. There will be no cost to you in the event of a study related injury, emergency care or hospitalization. If you seek emergency care for your child or if hospitalization is required at any time during the study or up to one month after your child takes the last dose of study medication, you should tell the treating doctor that your child is/was enrolled in this
research study. The National Institutes of Health, per policy, cannot provide compensation for research-related injuries.

APPENDIX XIII-A (Cont.)

WHAT ARE MY CHILD’S RIGHTS AS A RESEARCH SUBJECT?

Taking part in this study is completely voluntary. You may choose not to allow your child to take part in this study or to take your child out of the study at any time. Your child will still receive medical care no matter what you decide.

We will tell you about new information from this or other studies that may affect your child’s health, welfare or willingness to stay in this study. If you want the results of the study, let the study-staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above (24 hour accessibility)

For questions about your child’s rights as a research subject, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above
SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to allow your child to take part in this study, please sign your name below.

____________________________                ________________________________
Participant’s Name (print)   Participant’s Signature and Date

____________________________                ___________________________________
Participant’s Legal Guardian (print)  Legal Guardian’s Signature and Date
(As appropriate)

________________________                        ___________________________________
Study Staff Conducting              Study Staff Signature and Date
Consent Discussion (print)

________________________                          ___________________________________
Witness’ Name (print)     Witness’ Signature and Date
APPENDIX XIII-B

DIVISION OF AIDS

SAMPLE INFORMED CONSENT

PHARMACOKINETIC ANALYSIS OF ISONIAZID (INH)

IMPAACT P1041

A Randomized, Double Blind, Placebo Controlled Trial to Determine the Efficacy of Isoniazid (INH) in Preventing Tuberculosis Disease and Latent Tuberculosis Infection among Infants with Perinatal Exposure to HIV

Short Title: Efficacy of Isoniazid to Prevent Tuberculosis in Infants Perinatally Exposed to HIV

FOR CHILDREN AT CAPE TOWN AND DURBAN ONLY

WHY IS THIS STUDY BEING DONE?

You have agreed to allow your child to be in a study to see if isoniazid (INH) or placebo may prevent your child from becoming ill or infected with tuberculosis (TB). To help us determine whether or not the INH dose that we are using is the safest and that will work the best when given to children, some children will have repeat blood samples taken to measure the amount of INH in the blood. The dose used in this study is based on information obtained when adults took the drug. These blood tests will also help us to understand the differences in how children's bodies use INH as they get older. Although INH has been used successfully for the treatment of TB, there is little information about how the bodies of children, as young as three months and as old as 25 months, use INH to fight TB. Also, some children process INH in their bodies faster than other children, so the study will use a special test called an NAT-2 Genotype to find out how your child processes INH. The NAT-2 Genotype test is a laboratory test done on your child’s blood. In order to answer these questions, we are asking your permission to allow us to test the amount of INH in your child’s blood, as well as perform the NAT-2 Genotype test. You will be told of your child’s test results. If you decide not to allow your child to have these repeat blood samples taken, your child may still receive INH (or placebo).

WHAT DOES MY CHILD HAVE TO DO TO PARTICIPATE IN THIS STUDY?

If you allow your child to be in this study, your child will be randomly assigned (like the flip of a coin) to one of two groups. If your child is assigned to Group A, your child will have two blood
samples taken when s/he enters the study and two blood samples taken at the 21-month visit. These samples will be taken at 1 and 3 hours or at 2 and 4 hours after

APPENDIX XIII-B (continued)

your child takes his/her dose of INH. If your child is in Group B, your child will have two blood samples drawn at the 3-month visit and two blood samples drawn at the 21-month visit. These samples will be taken at 1 and 3 hours or at 2 and 4 hours after your child takes his/her dose of INH. If possible, your child should not have anything to eat (including milk) one hour before and two hours after taking INH because food may decrease the amount of INH in the blood. About 2 ml of blood (half a teaspoon) will be drawn for each blood sample for a total of about 4 ml of blood (or one teaspoon).

HOW LONG WILL MY CHILD BE IN THIS STUDY?

Your child will be in this study until he/she is 24-25 months of age.

HOW MANY CHILDREN WILL PARTICIPATE IN THIS STUDY?

About 336 children will be in this study.

WHAT ARE THE RISKS OF PARTICIPATING IN THIS STUDY?

Blood drawing may cause some pain, discomfort, bleeding, or bruising where the needle enters the skin. Blood drawing may also cause a lightheaded feeling, and in rare cases, fainting. A small blood clot may form at the site where the needle enters the skin or swelling of the surrounding skin may occur. There is also a small risk of a minor infection at the blood draw site.

ARE THERE ANY BENEFITS TO TAKING PART IN THIS STUDY?

Your child will receive no benefit from taking part in this study but the results may indicate that a different dose of INH is needed to work effectively or may help us understand what doses of INH are best used in other children.
SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to allow your child to take part in this study, please sign your name below.

All other information that is contained in the main study consent you signed also applies to this addendum consent as well.

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<th>Participant’s Name (print)</th>
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