Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Menzies D, Adjobimey M, Ruslami R, et al. Four months of rifampin or nine months of isoniazid for latent tuberculosis in adults. N Engl J Med 2018;379:440-53. DOI: 10.1056/NEJMoa1714283

ON-LINE SUPPLEMENT – Original and Final Study Protocols, and Statistical Analysis Plan

"This supplement contains the following items:

1. Original statistical analysis plan, and, summary of changes

2. Original protocol, final protocol, and summary of changes.

STATISTICAL ANALYSIS PLAN

RCT OF 4RIF VS 9INH: PLANNED HAS BEEN ABSTRACTED FROM ORIGINAL PROTOCOL, WITH DESCRIPTION OF CHANGES (CHANGES NOTED IN BOLD FONT)

ADULTS:

DATA CLEANING & CHECKS

The minimum, maximum, and mean values of all continuous variables will be verified. Histograms will be constructed to examine the distribution of important variables such as age.
The distributions of binary and categorical variables will be examined.
No Change

COMPARISON OF TREATMENT ARMS

Subjects will be compared on the basis of the following variables according to the treatment arm they were randomized to. Risk factor variables were chosen because they modify the risk of developing active TB. No statistical tests will be conducted.
Demographics: Centre, Age (Mean , SD) and in categories as: 18-35, 36-50, and 51-90, Sex,
Body size: Height (median, 25^{th}, 75^{th} percentiles), Weight (median, 25^{th}, 75^{th} percentiles), BMI (median, 25^{th}, 75^{th} percentiles),
Risk Factors: TST reaction size in categories: 5-9, 10-14, $\geq$15 mm, HIV infection, Close contact of a patient with confirmed active TB, Casual contact of a patient with confirmed active TB, Immune suppressive conditions (diabetes, TNFa inhibitor therapy, Renal failure), Upper lobe fibronodular disease with area >= $2cm^2$, Combination of risk factors, including: (i) Born in or living in country with TB incidence >100/100,000; (ii) Aboriginal Canadian living on reserve; (iii) BMI < 19; (iv) Abnormal CXR consistent with past TB-infection (Upper lobe fibronodular disease with area <2 cm^2 or granulomas, or calcified hilar lymph nodes, or costo-phrenic angle blunting, or apical cap).
Chest Xray Result: Categories of normal, Apical/upper lobe fibronodular disease $\geq$2 cm^2, Apical/upper lobe fibronodular disease <2 cm^2, Granulomas, Costophrenic angle blunting, Hilar lymph node enlargement, Other possible TB-related, Abnormal, but not TB related
Clustering: number of households, average household size, and in categories of 1, 2,3, 4+.

Change: As per the request of the statistical reviewer, and policy of the NEJM, we performed testing for significance of differences (t-tests if normally distributed and Wilcoxon if not normally distributed, or chi-square, depending on type of variable) between those randomized to the two arms in terms of the baseline characteristics (age, sex, BMI, TST reaction size, HIV, Close contact, casual contact, immune suppressive condition) of the two arms and reported these using asterisks in Table 1.

ANALYSIS GROUPS

We plan to analyze the study population in two ways:
Modified Intention to Treat (MITT): subjects randomized but excluding those who withdrew consent, or were close contacts of INH- or RIF- resistant TB cases. Subjects who were lost to follow-up contributed follow-up time up to the moment when they were last contacted.
Per Protocol: subjects randomized who completed 80% or more of doses within the allowed time. This was 120% of duration if all doses were taken consecutively – meaning 146 days for 4RIF and 324 days for 9INH.
No Change

MISSING DATA

A complete case approach will be followed given that we anticipate complete data for most subjects. A person-time analysis will be used to avoid excluding subjects lost to follow up, though we anticipate low rates of loss to follow up.
NO CHANGE:

PLANNED PRIMARY DATA ANALYSIS – IN ADULTS

The primary study outcome will be the occurrence of microbiologically or histologically confirmed active TB, confirmed by the majority of the 3-member independent clinical review panel. The primary analysis, comparing the rate of occurrence of active TB per patient-year will be performed with the use of an unadjusted Poisson marginal model fitted using generalized estimating equations (GEE) to allow us to take clustering by household into account [193,194]. We will use a log link and assume an exchangeable correlation structure. Robust standard errors will be used. The log of follow up time will be used as an offset in the regression model, which will allow us to account for differing lengths of follow up time [194] If clustering is significant we will calculate a rate ratio from the GEE Poisson regression. But if the effect of clustering is negligible, as anticipated, the proportion of subjects randomized to both treatment groups developing confirmed active TB, and the associated 2-sided 95% confidence interval for the difference, will be estimated, using an incidence density method, expressed as TB events per 100 person-years of follow-up. This will allow us to include information from subjects who are followed for some time before being lost to follow-up.

Rate difference estimated using Poisson regression with log link and using log person time as an offset and to account for clustering we used a GEE method for this estimation. The rate difference was estimated using the method described in reference 24 in the manuscript. Rate differences were calculated for confirmed active TB, and for all TB (confirmed and clinically diagnosed).
Clarification: Because active TB could be diagnosed up to the end of the 28th month of post-randomization follow-up and still counted as occurring within the allowed follow-up time, follow-up was continued to the end, or even past 28 months in many Phase 3 participants. In these participants, follow-up months were truncated to 28.9 months. In Phase 2 follow-up was continued until 36 months post randomization. For Phase 2 participants, person-years of follow-up was based on follow-up to a maximum of 28.9 months (ie also truncated to 28.9 months).

In secondary analyses, if covariates are unbalanced between treatment arms, we will adjust for important covariates (age, sex, etc). We will estimate adjusted Poisson marginal models via GEE using a log link and exchangeable correlation structure, and including an offset as above. We will report adjusted rate ratios with 95% confidence intervals.

Not performed, as no serious imbalance was noted.

Non-Inferiority analysis:

We have assumed 4RIF efficacy of 90%, based on available evidence. As shown below, if 50% of the 2,898 randomized to each group complete therapy and 28 months follow-up, this would provide more than 90% power, to confirm **non-inferior** efficacy of 4RIF, if the **non-inferiority** margin was 25% - equivalent to a minimum efficacy of 4RIF of 65%. (In other words, we would declare 4RIF **non-inferior** to 9INH if the efficacy of 4RIF was not more than 25% worse than 9INH.) This efficacy has been considered sufficient for authoritative recommendations of 6INH [107, 138], which has had efficacy of 40-69% in trials [33,61,65,68,104,128].

Table S2: Sample size to assess non-inferiority of 4RIF efficacy
(calculated using alpha=0.05 and one-sided test using methods suggested by Blackwelder [180])

Expected cumulative incidence of TB		Tolerated difference		Number (per group) required to provide power of	
Untreated	**9INH**	**Δ (25%)**	**Maximum Event rate with 4RIF**	**80%**	**90%**
3%	0.3%	0.75%	1.05%	658	911
4%	0.4%	1%	1.4%	493	683
5%	0.5%	1.25%	1.75%	394	546

No change – we compared the upper bound of the observed rate difference (4RIF – 9INH) for all cases of active TB (confirmed and clinical) among participants who completed assigned study drug per protocol to the tolerated difference of 0.75% (cumulative over 28 months of follow-up) which would correspond to 0.32 per 100 person-years.

We also calculated the rate difference that would have occurred in the per protocol analysis if 4RIF had 65% efficacy, 9INH had 90% efficacy and the expected rate of disease in untreated was based on the observed rate in those who completed 9INH per protocol. In that case the non-inferiority margin was 0.32%, based on the lower rates that we observed.

INTERIM ANALYSES AND STOPPING RULES FOR ADULT

Primary outcomes

Only one interim analysis of the primary outcome will be performed, one year after 33% of patients have been randomized. Further interim analyses, such as one year after 67% of subjects have been randomized, will fall too close to the end of randomization, to have any meaningful impact. We wish to avoid falsely concluding that one regimen has significantly superior effectiveness with this interim analysis – a well-known risk. Hence, we will use a threshold of a p value <.001196, before concluding that 4RIF is significantly inferior to 9INH, and stopping the trial early.

This was not done; the overall event rate was so low, at the point in time that the analysis should have been done, that the DSMB felt this interim analysis was not necessary.

Serious adverse events (SAE)

Four months after randomization of 25%, 50%, and 75% of subjects, interim analyses will be performed of SAE. The DSMB will consider stopping the trial early if SAE rates are significantly higher with 4RIF. To balance the risk of unnecessarily stopping the study with interim analyses, with the need to ensure patient safety, we will use the method of Pocock, or an interim stopping level (p value) of 0.018 for each analysis.

This was done; on all three occasions, the DSMB concluded there was no indication of excessive AE in either arm, so they recommended to continue enrolment.

PLANNED SUB-GROUP AND SECONDARY ANALYSES

Active TB in subjects who complete treatment (efficacy)

Non-completion of therapy is obviously not a random event, and may be associated with characteristics that are associated with risk of disease[166]. Therefore we will compare the characteristics of compliant and noncompliant subjects in each group (see variables listed in "Comparison of Treatment Arms"), and use logistic regression to estimate efficacy, adjusted for important covariates.

Change made – Event rates were very low – so adjustment was not performed. The event rates were analyzed in the same way as the primary outcome was analyzed.

Confirmed and probable active TB

In secondary analysis we will combine these two outcomes, and use the same methods to compare rates of TB with both regimens, as described above for the primary analysis.

No change.

Subgroup according to study

We will perform the same analyses described in 2.18.2 and 2. 18.1 and the primary analysis in the subgroup of patients who were randomized in this study (excluding subjects who were randomized as part of our earlier phase 2 safety trial)

No change.

Treatment completion

We will compare the proportion of patients in each arm who:

- took at least 80% of doses (total completed)
- took at least 80% of doses within the allowed time (per protocol: 146 days for 4RIF, and 324 days for 9INH)
- took at least 80% of doses but not within the allowed time (completed, but not per protocol)
- did not complete for all reasons (took less than 80% of doses)

- did not complete due to death
- did not complete due to never starting
- did not complete due to Grade 1-2 AE or Grade 3-5 AE
- did not complete due to Grade 3-5 AE adjudicated as possibly or probably related to study drug
- did not complete due to patient decision to stop drug
- did not complete – but took 50-79% of doses, or 1-49% of doses.

A binomial model with identity link, accounting for correlation of patients from the same family (via generalized estimating equations using an exchangeable correlation structure and robust standard errors) will be used to estimate the risk difference between subjects randomized to 4RIF and those randomized to 9INH and 95% confidence interval in each case.

Change made: On the basis of comments from the Statistical Reviewer, we present a confidence interval and p-value for the two first comparisons. The other completion measures are presented descriptively only, as risks and risk differences.
Clarification: In Phase 3 pill counts were the main basis of determination of adherence and completion of study drug. These were recorded on every treatment phase follow-up visit. Phase 3 participants were considered to have completed per protocol if the number of pills documented to have been taken, represented more than 80% of total doses and within allowed time. In Phase 2, Medication Event Monitoring Systems (MEMS@) were used to assess adherence and determine study drug completion. Phase 2 participants were considered to have completed per protocol if the number of pills taken, as recorded by the MEMS system, represented more than 80% of total doses and within allowed time. However, when the MEMS were not available (lost, forgotten, etc), or not functioning (broken) we relied on verbal self-report of pills taken. Phase 2 participants were considered to have completed therapy but not per protocol if: they took at least 80% of doses, but in longer than allowed time; or, they took less than 80% of doses based on MEMS documentation, but based also (or exclusively) on self-report had taken more than 80% of doses in total. Patients who had taken less than 80% of doses – as recorded using any method – were considered to have not completed study therapy

Adverse events (AE)

This outcome will be defined as the occurrence of Grade 1-2 AE, or Grade 3-5 AE, based on the Grading by the majority of the independent review panel. Differences in all Grade 3-5 AE, Pregnancies, and Grade 3-5 hepatotoxicity, as well all Grade 3-5 and Grade 3-5 hepatotoxicity AE that the panel judged to be possibly or probably related to study, between the two groups will be tested with Chi-squared or Fisher's exact tests. Sub-group analyses will compare rates of SAE by age, sex and HIV status. Drug interactions with 4RIF are common, and of particular interest since this will be the largest trial with mono-Rifampin therapy. All analysis of SAE and drug interactions will be presented as exploratory, with appropriate caution [166,196]. However in view of the very limited published experience with 4RIF, any information about adverse effects, including drug interactions, would be very useful for clinicians.

We will compare:

- All AEs where drug stopped, and reviewed by AE panel
- Grade 1-2 and 3-4 AEs where treatment not discontinued (ie started, but restarted successfully)
- Grade 1-2 AEs and treatment discontinued
- Grade 3-5 AEs – overall and those judged probably or possibly related to study medications
- Deaths judged probably or possibly related to study medications
- Specific Grade 3-4 AEs if numbers permit (eg hepatotoxicity).

Change made – we estimated risk of Grade 1-5 adverse events (AE) by arm – and estimated risk differences, also adjusted for potential clustering as we felt this was a more conservative approach. We used a binomial model with identity link, accounting for correlation of patients from the same family using an exchangeable correlation structure and robust standard errors (via generalized estimating equations). The comparison of rates of AE within subgroups defined on the basis of age, sex, HIV status, etc – has not been done yet, although this is planned. If there were 0 events in an arm this caused the GEE to fail. For these comparisons, we presented a CI based on the method of Newcombe (Newcombe, RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med. 1998;17(8):873-90).). To assess

robustness to clustering we also estimated the confidence interval by taking only the first person from every household – (this is the most conservative method – and results were the same).
In addition, in response to concerns re differential ascertainment of AEs because of the different lengths of treatment, we conducted a secondary analysis to compare rates of AEs that occurred within the first 146 days of treatment (representing the maximum allowable time to complete 4RIF).
We defined participants as not exposed to study drug, if there was no documentation of pills taken – measured during treatment phase follow-up visits. We also considered that participants were considered exposed to study drug - even if they did not return for any treatment phase follow-up visits, and so had no documented pills taken, BUT had reported verbally having taken any study drug, as this information was specifically sought and recorded in end-of-treatment case report forms.

Drug resistance among cases of active TB after randomization

The occurrence of drug resistant TB after LTBI therapy may result from selection of drug resistant mutants during therapy (acquired resistance), or because they were infected with drug resistant organisms (primary resistance). All patients with positive cultures for M Tuberculosis within 28 months for adults and until 16 months for children after randomization will have drug sensitivity testing. Adequate facilities are available in all sites. If drug resistant tuberculosis is found, patients will be placed on appropriate therapy and followed by the site investigators in collaboration with the local TB programs. We will compare the prevalence of resistance to INH and RIF among study participants who develop active TB. The prevalence of underlying (or primary) INH resistance in the community will be assumed as the prevalence in those who took 4RIF, and of RIF resistance in those on 9INH. Acquired RIF resistance will be taken as the difference in prevalence of RIF resistance between subjects on 4RIF and 9INH. Power to detect significant differences will be limited, unless drug resistance is very common, but descriptive information on any excess resistance would still be useful.

No change. Occurrence of drug resistance described in text.

Cost-effectiveness of the two regimens

We will have detailed information on all health system activities – including scheduled visits, therapy and tests as part of routine follow-up, unscheduled visits, tests and therapy for adverse events, and diagnostic and therapeutic activities for the cases of active TB that develop. These will be valuated using local costs for all such activities (for details see RCT Appendix 4). We will also have measured incidence of active TB among subjects randomized to the two LTBI regimens. If one arm is cheaper and associated with fewer TB cases then it will be clearly preferable from a cost-effectiveness standpoint. If one arm is more expensive but associated with fewer TB cases then we will calculate the incremental cost per additional TB case prevented by the more effective (but more expensive) regimen. In primary analysis we will use average Canadian costs to evaluate all health care activities, but in secondary analyses will perform the same analyses using costs from each site. This will be of particular interest in lower-income countries, where LTBI therapy is considered a low priority [158,199]. In sensitivity analyses we will vary the costs for active TB, given that the cases detected in this study will likely be at an early stage, so may underestimate average costs. We will also vary the costs of rifampin, which is extraordinarily expensive in Canada, compared to international prices[126].

Not yet performed. Planned analysis

CHILDREN:

DATA CLEANING & CHECKS

The minimum, maximum, and mean values of all continuous variables will be verified. Histograms will be constructed to examine the distribution of important variables (age, etc).
The distributions of binary and categorical variables will be examined.

COMPARISON OF TREATMENT ARMS

Subjects will be compared on the basis of the following variables according to the treatment arm they were randomized to. Risk factor variables were chosen because they modify the risk of developing active TB. No statistical tests will be conducted.
Demographics: Centre, Age (Median , 25th and 75th percentiles) and in categories as: 0-4, 5-12, 13-17, Sex,
Body size: Height (median, 25th, 75th percentiles), Weight (median, 25th, 75th percentiles), BMI (median, 25th, 75th percentiles),
Risk Factor: TST reaction size in categories:<5, 5-9, 10-14, ≥15 mm, Reason for eligibility (HIV infection, Close contact of a TB case,

TST>15 and from endemic country), Chest Xray Result: Categories of normal, non-TB abnormality, Hilar Lymph note, Other possible TB related
Clustering: number of households, average household size, and in categories of 1, 2,3, 4+.

Change Made: As per the request of the statistical reviewer, and policy of the NEJM, we performed testing (t-tests or chi-square, depending on type of variable) between the baseline characteristics (age, sex, BMI, TST reaction size, HIV, Close contact, casual contact, immune suppressive condition) of the two arms and reported these using asterisks in Table 1.

ANALYSIS GROUPS

We plan to analyze the study population as follows:
Modified Intention to Treat (MITT): as randomized, excluding subjects who were randomized but had a negative second TST eight week post exposure as described in the protocol
Per Protocol: subjects who completed at least 80% of doses within the allowed time

PRIMARY - Adverse events:

This outcome will be defined as the total of Grades 1-5 AE, that are diagnosed as probably related to the study drug by at least two of the three-member independent review panel. Following the method described by Kaul, we will declare 4RIF not inferior to 9INH in terms of AEs if the lower bound of the confidence interval around the difference in proportion of subjects experiencing an AE excludes the limit specified in section 2.11.1. The confidence interval will take potential clustering into account. We will estimate the risk difference and 95% confidence interval via a logistic regression with binomial distribution and identity link, and assuming an exchangeable correlation structure with robust standard errors.
Further descriptive analysis of AE including differences in type and severity of AE will be presented as exploratory, with appropriate caution. This analysis is justified by the very limited experience with 4RIF in children, making any information about tolerability and adverse effects of value.

No change; except minor symptoms were also analysed descriptively. To account for the differences in the number of follow-up visits due to the different lengths of treatment, we estimated this as the proportion of all visits at which symptoms were reported. No serious adverse events attributed to treatment were reported in either arm. We estimated a 95% CI around the risk difference of 0 using the score based method of Newcombe. ("Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med. 1998;17(8):873-90.") To account for clustering we took the extreme view that additional children from the same household contributed no additional information (i.e. we took only one child from each household).

PLANNED SECONDARY ANALYSES IN CHILDREN:
Treatment completion, Active TB, and Drug resistance among cases of active TB after randomization (as described for adults).

No change: Differences between arms in rates of active TB (estimated per 100 person years), and risk of treatment completion adjusted for potential clustering effect. Occurrence of drug resistance – descriptive only.

INTERIM ANALYSES AND STOPPING RULES FOR CHILDREN
Primary Outcome: Serious adverse events (SAE)
Four months after randomization of 25%, and 50% of subjects, interim analyses will be performed of SAE. The DSMB will consider stopping the trial early if SAE rates are significantly higher with 4RIF. To balance the risk of unnecessarily stopping the study with interim analyses, with the need to ensure patient safety, we will use the method of Pocock, or an interim stopping level (p value) of 0.018 for each analysis.

Change made: No serious events occurred through-out the paediatric trial. Therefore, at the two timepoints, no analyses could be done. The DSMB recommended to continue enrolment.

ORIGINAL STUDY PROTOCOL (VERSION 1, March 30, 2009)

A randomized clinical trial of 4 months Rifampin vs. 9 months Isoniazid for latent TB infection. – Phase 3 effectiveness (Version 1: March 2009)

SECTION 1 THE NEED FOR A TRIAL

1.1. WHAT IS THE PROBLEM BEING ADDRESSED?

1.1.1 Importance of tuberculosis (why study TB?)

1.1.1.1 Globally: As a disease, tuberculosis (TB) is unique in that it is wholly preventable and treatable, yet on a global scale, incidence and mortality continue to rise [1]. The World Health Organization (WHO) has estimated that there are between 8 and 9 million new cases each year [2], that 200 million persons alive today will develop active TB during their lifetime [2], and that 30 million will die from TB over the next decade [2] - equivalent to the population of Canada. TB remains the world's most important infectious cause of morbidity and mortality among adults, yet remarkably, many consider this disease to be of little current significance or importance [3]. In many industrialized countries, after decades of decline, incidence of tuberculosis increased in the 1980's. In most, with greater investment in TB control, rates have since levelled off or declined somewhat [4,5] although in some, such as Britain [6], incidence continues to increase.

1.1.1.2 TB in Canada (why study TB in Canada?): Incidence of active TB in Canada declined steadily from the beginning of the 20th century until the mid 1980's. Since then, the number of new cases reported each year has remained largely unchanged. In 2006, overall incidence was 5 new active cases per 100,000 population [7], but rates were substantially higher in certain populations and regions, because TB remains a disease of poor and marginalised populations [8-10]. Incidence among aboriginals ranges from 25 to 50 per 100,000 compared to less than 3 per 100,000 among non-aboriginal Canadian-born [7]. Rates among the foreign-born are three times higher than the national average [7]; 90% [11-14] of cases among foreign-born arise because of reactivation of dormant TB infection acquired before immigration, in their countries of origin [15-17].

1.1.2 Pathogenesis of tuberculosis (why treat latent TB infection?)

TB infection is transmitted by the airborne route from patients with active pulmonary TB disease. In more than 95% of individuals who acquire primary infection, there is no clinical illness and the TB bacilli enter a latent or dormant state; this may last only six months or lifelong. Latent TB infection (LTBI) causes no symptoms, and is not contagious. Usually the only detectable abnormality is a positive tuberculin skin test. The World Health Organization has estimated that close to **two billion people** have LTBI [2,3], of whom approximately 10% will reactivate over their lifetime. Therapy can reduce the likelihood of future active disease, but is inefficient, because the subgroup that will develop disease can not be distinguished from the majority who will not, although some have recognized risk factors (RCT Appendix 1: Table 1) [18-53].

1.1.3 Importance of therapy of latent TB infection (how many are treated?)

In a recently completed survey, we found that more than $25 million is spent annually in Canada for the diagnosis and treatment of approximately 20,000 persons with latent TB infection in 2006[54]. One survey of 110 US health departments found that 127,996 persons initiated LTBI therapy between 2000-2002 [55]. Based on this, and a second survey of 37, 857 patients in 244 U.S. health departments, it has been estimated that 290,000- 433,000 persons are treated annually for LTBI in the US [56], of whom more than 80% receive Isoniazid daily for 9 months (9INH) [57]. This data and reports from other countries [58-60] indicate that LTBI therapy is a major component of TB control in many high income countries. In low and middle income countries the World Health Organization has recommended LTBI therapy for close contacts, especially children, of patients with smear positive pulmonary TB, and is promoting expansion of use of INH for HIV infected persons with LTBI.

1.2 THE PRINCIPAL RESEARCH QUESTIONS

Hypothesis: Therapy of latent TB infection (LTBI) with four months of daily Rifampin (4RIF) will result in cumulative incidence of microbiologically confirmed active TB during 28 months following randomization, that is significantly lower than the cumulative incidence of active TB among participants randomized to nine months of daily isoniazid (9INH).

Primary objective: To compare the cumulative incidence during 28 months after randomization, of confirmed active tuberculosis (TB) among all persons randomized (effectiveness, using intention to treat analysis) to 4RIF and 9INH.

Secondary objectives:

(i) Compare the cumulative incidence of confirmed active TB among those who took at least 80% of doses of the LTBI treatment to which they were randomized, in less than 120% of the allowed time (i.e. ***efficacy***).

(ii) Compare the cumulative incidence of ***probable***, as well as confirmed active TB between patients randomized to the two regimens during 28 months following randomization.

(iii) Compare rates of ***Grades 3&4 adverse events*** during treatment between subjects randomized to the two regimens.

(iv) Compare ***health system costs, and cost-effectiveness*** of the two regimens, in the different sites.

(v) Describe occurrence of ***drug resistance*** (to INH or RIF) among subjects who develop confirmed active TB.

1.3 WHY IS A TRIAL NEEDED NOW?

1.3.1 History of preventive therapy ("the INH story") (see RCT Appendix 1 Tables 2&3) [61-73]

In the 1950's and 1960's, a number of large scale placebo-controlled randomized clinical trials of Isoniazid (INH) therapy for LTBI were conducted. In trials where high compliance was achieved, or in subgroup analysis of "completer-compliers" 12 months of INH resulted in risk reductions of 83-93%, relative to placebo [63,65,67]. About 50,000 subjects received INH, yet adverse events, including hepatitis were uncommon, and there were no deaths related to drug induced toxicity in these studies [61,62,64]. As a result of this evidence of efficacy and safety, in 1971 the American Thoracic Society (ATS) issued recommendations strongly encouraging INH therapy of LTBI [74]. The resultant widespread use of INH was quickly followed by the widespread occurrence of hepatitis - with fatalities[75 69 76], which, together with apparently contradictory risk benefit analyses [77-81], resulted in widespread doubts about the benefits, and revised recommendations for use of INH[82-84].

Publications in the past decade have reported much lower rates of hepatotoxicity [70,72,73,85] and mortality [70-72]., which may be due to better selection of candidates for therapy, or closer follow-up. Recent analyses have concluded that INH therapy will be of benefit and cost-effective for healthy infected persons without risk factors, and be very beneficial and cost-effective for infected persons at higher risk of disease [86,86,87,87-91]. However the overall effectiveness of INH remains low – because of physician under-prescription [92-96] (fearing serious side-effects), and poor patient compliance (because of the long duration) [70,93,96-102], and costly[94]– because of the close monitoring required to detect potential serious, even fatal, adverse events.

1.3.3 Alternates to INH for therapy of LTBI (RCT Appendix 1: Tables 4&5)

These problems of INH therapy have stimulated substantial interest in the evaluation of shorter regimens for LTBI.

1.3.3.1 Rifampin and Pyrazinamide ("The RIF-PZA story" – history repeats itself): Based on initial animal studies [103] and randomized trials among HIV infected [104-106] the regimen of 2 months daily Rifampin & Pyrazinamide (2RIF-PZA) was recommended in 2000 [107]. This was soon followed by reports of severe and fatal drug induced hepatitis [108-111]. In subsequent studies, serious adverse events, particularly hepatitis were significantly higher among patients given 2RIF-PZA than in patients given INH [58,112-115 116 55,112,115,117,118], despite close monitoring. Interestingly, default rates [113,115,117,119] and costs [113,117] of 2RIF-PZA were the same, or higher than 9INH in several studies. As a result this regimen has been almost totally abandoned [120].

1.3.3.2 - Rifampin alone: The only published randomized controlled trial with a mono-RIF regimen compared 3 months RIF, 6 months INH, 3 months INH-RIF and placebo. Interestingly, the 3RIF regimen had efficacy of 63%, which was superior to all other regimens, and no hepatotoxicity [33]. 6 months RIF was given to 157 high school contacts[121], and 49 homeless contacts [122] - of INH-resistant cases. In both series 6RIF was well tolerated, with no subsequent case of active TB. Further evidence of the high efficacy, despite much shorter therapy comes from experience with active TB. In trials with head to head comparisons, addition of RIF allowed the total duration of therapy to be halved [158,167]. Hence, implicit in our study hypothesis is that efficacy of 4RIF is 90% - the same as 9INH. Several observational studies of LTBI therapy provide further evidence of the advantages of 4RIF. In the first, of 1,379 patients on 4RIF, 1 (0.1%) developed hepatitis, and 987 (72%) completed therapy, compared to 12 (2%) with hepatitis and 405 (52%) completion among the 770 who started 9INH [123]. In the second, 261 subjects initiated 4RIF, of whom 210 (81%) completed therapy, and 8 (3%) developed SAE (no hepatitis), compared to 113 (53%) completing, and 13 (6%) with SAE (3 with hepatitis) of the 213 who started INH [124]. In a third study, of 749 given 4RIF, 76% completed and 9 (1.2%) developed SAE (3 = 0.4% with hepatotoxicity)[125]. The past problem of higher cost of RIF has been resolved by dramatic price reductions in the international market [126]. There has been no emergence of INH resistance following INH therapy of LTBI in several large studies [62 33,65,127], nor emergence of Rifampin resistance following RIF therapy [33,122,123,128] except for one case-report of a patient who was very poorly compliant with therapy [129]. Rifampin containing regimens would also be more cost-effective than 9INH for LTBI treatment in immigrants from countries with high rates of INH resistance [130]. In summary, mono-RIF therapy can be effective for LTBI, may be more cost-effective[130], with better completion rates [131], and less hepatotoxicity [33,121-124].

1.3.3.3 INH and Rifampin (INH-RIF): In the Hong Kong study, the INH-RIF combination was the most toxic, and least effective of the three active regimens [33]. A large uncontrolled paediatric case series utilizing regimens of 6, then 4, then 3 months of daily INH-RIF, reported few cases of TB among those treated, although community rates were used for comparison[59]. A recent questionnaire survey reported that 3 of 344 paediatric household contacts treated with 3INH-RIF developed active TB – a rate of 8.7/1,000 [60] . This was 48% less than the rate of TB among similar subjects who received placebo in earlier trials [62]. In Uganda, 3 months daily INH-RIF-PZA was less effective than 6INH, with similar completion rates, and 4 times higher SAE [128]. In Saskatchewan twice weekly directly observed INH-RIF was well tolerated, with higher completion rates, and lower subsequent TB incidence than patients given 12 months INH [132]. In three recent trials, completion of 3-4 months of INH-RIF was better than 6INH, with similar adverse events and efficacy [133-135]

1.3.3.4 INH-Rifapentine (INH-RPT): RPT is a new Rifamycin with a half life five times longer than Rifampin that can be given once weekly. In a recent randomized trial, 2 of 206 (1%) TST positive household contacts receiving 3 months of directly observed once weekly RPT-INH developed Grade 3 or 4 hepatitis, compared to 20 of 193 (10%) of subjects who received 2RIF-PZA [136]. Active TB developed in 3 (1.5%)

who received 3INH-RPT, compared to one (0.5%) of the 2RIF-PZA group. A large CDC-sponsored trial comparing 3INH-RPT and 9INH is nearing completion. However RPT is an "orphan drug" produced in limited quantities by the manufacturer, that has had poor results in treatment of active TB [137]. Its utility may be limited because of the need for direct observation - impractical for private providers, and increasing costs.

1.3.4 Current Canadian[138], and American [107] recommendations for LTBI therapy

Until 1999, 12INH was the standard of care in North America, although 6INH was considered acceptable, because the superior completion rate was considered to offset its lower efficacy [139]. However, based on an analysis by Comstock [140], both the ATS and CTS published revised guidelines in 2000, recommending that 9INH should be the standard of care for LTBI therapy, given its 90% efficacy [140]. 6INH, 2RIF-PZA, and 4RIF were recommended as alternatives.

1.4 RESULTS FROM SYSTEMATIC REVIEWS, AND META-ANALYSES

Several extensive reviews [62] and meta-analyses of trials in HIV infected [141] and uninfected [142] persons have concluded that 6-12 months INH has significantly better efficacy than placebo – in the populations we plan to study. A recent meta-analysis of 5 trials involving a total of 1926 adults randomized to 3INH-RIF or INH concluded that the rate of active TB was similar (4.2% vs. 4.1%) as was the rate of SAE (4.9% vs. 4.8%) [143]. However 83% of subjects received 6INH, which has efficacy of only 40-70% [33, 61, 65, 68, 104, 128]. Meta-analyses of 2RIF-PZA have been published [118] but this regimen has been abandoned. There are no published meta-analyses of 4RIF, as there are no trials with 4RIF, and only 1 trial with 3RIF [33].

1.4.1 Summary of current evidence

Each year, more than 20,000 persons in Canada [54], and at least 300,000 persons in the US [144] initiate LTBI therapy. Over 80% are prescribed 9INH [57], which is considered the standard of care [107, 138], but is lengthy, costly, may cause serious adverse events, and has poor completion [70, 93, 96, 98-102]. Of the available recommended alternative regimens, 6INH has efficacy of only 40-70%, and similar risk of adverse events, The 2RIF-PZA regimen was enthusiastically adopted, but then abandoned due to unacceptable toxicity [108, 109, 112, 117, 145]. 3INH-RIF appears to have similar efficacy as 6INH, but greater toxicity. This leaves 4RIF, for which there is limited efficacy data, but consistent evidence that safety and compliance are better than with 9INH. With CIHR funding we have completed two trials to compare the 4RIF regimen with 9INH (Section 2.19, and manuscripts in Research Module Appendix). In these and other studies, 4RIF had better compliance and completion rates [123, 124, 131], lower costs [146, 147], and better safety [123, 146], particularly less hepatotoxicity [148] - the most serious complication with INH.

1.5 HOW WILL THE RESULTS OF THE TRIAL BE USED?

TB is a major global pandemic, and persists in Canada among impoverished and marginalized groups such as urban poor and aboriginal Canadians, as well as immigrants and refugees. Treatment of LTBI has individual and public health benefits, but the current standard of care - 9INH - has serious side effects, and the length of therapy increases costs, yet reduces compliance [93, 97-99, 149], and thereby effectiveness. The benefits of a shorter, cheaper, and safer treatment for LTBI would be substantial [130, 150, 151]. To date, we and others have found that 4RIF has significantly better completion rates [123, 131], lower costs [123, 146], and better safety [123, 146]. The proposed study will be the largest trial to evaluate mono-RIF therapy for LTBI, and the first trial to evaluate the currently recommended 4 month RIF regimen. Thus, our proposed study will provide urgently needed data [152, 153] on effectiveness and efficacy of this already recommended (and utilized) alternative LTBI therapy. Involvement of international as well as sites across Canada will provide valuable information on feasibility, tolerability, safety, costs, and effectiveness of 4RIF in different settings and patient populations - enhancing the potential applicability of results.

1.6 RISKS FOR THE TRIAL PARTICIPANTS

Available evidence, from *millions* of patients treated for active TB with RIF in combination with other drugs [154, 155], suggests that RIF is well tolerated and safe. Hepatitis is the most important and potentially fatal complication of INH and RIF-PZA therapy of LTBI. On the other hand, mono-therapy with RIF has been associated with very low rates of hepatitis – in Phase 1&2 [131, 148], and elsewhere [33, 121-124] (RCT Appendix 1 - Table 5). Nevertheless, in view of the 2RIF-PZA experience, where initial trials suggested excellent safety, but unacceptable toxicity was seen with introduction into routine practice, it is prudent to continue to closely monitor the safety of 4RIF. This will be done by independent review of all possible Grade 3 or 4 SAE, plus periodic interim analyses of safety during the trial. Patients will be carefully questioned regarding concomitant medications, to identify potential drug interactions, and will be excluded if these can not be managed easily. Mono-therapy of patients with unrecognized active TB, of particular concern in HIV infected patients, may lead to drug resistance, which has serious implications for treatment[152, 153]. In all settings we will ensure that study participants have access to all necessary investigations to exclude active TB, including cultures. In some settings this means budgetary allocations to pay for these investigations if necessary. In the long term, 4RIF will not be useful in resource-limited settings if extensive investigations are needed before starting therapy. Hence, we will develop and evaluate low cost diagnostic algorithms to identify candidates for LTBI therapy likely to have active TB, for use after the trial (RCT Appendix 5). Initial or primary drug resistance can render LTBI therapy ineffective [127]. However rates of RIF resistance rates are very low and much lower than INH resistance in all participating countries (RCT Appendix 1 - Table 6) [156], [157], enhancing the rationale and ethical

acceptability of evaluating 4RIF. INH is recommended unless prevalence of primary drug resistance exceeds 50% [107,158] - clearly not the case in any country, or if the subject is a contact of INH resistant cases INH [107,138] – an exclusion criteria for this trial. Finally, subjects will be followed two full years after completing 4RIF to ensure early detection of active TB if this therapy fails.

SECTION 2 THE PROPOSED TRIAL

2.1 STUDY DESIGN

We propose a multi-centre randomized 2-arm positive control open-label clinical trial. Patients prescribed standard therapy for LTBI (i.e. 9 months INH) will be approached to participate before they begin therapy. After providing informed consent, subjects will be registered using a web-based system, accessible at all times. This system will verify eligibility and perform immediate on-line randomization, using a computer generated random sequence, in equal numbers to 4RIF or 9INH - both daily and self administered. Randomization will be stratified by site and in blocks of variable length. The primary end-point will be the occurrence of microbiologically confirmed active TB. Secondary end-points include occurrence of confirmed and probable active TB, Grade 3 or 4 adverse events (SAE), drug resistant active TB, and health system costs. All primary outcomes will be reviewed by an independent 3-member clinical review panel, and all SAE will be reviewed by a different independent 3-member panel. Both panels will be blinded to the study drug, and patient identity.

2.1.1 Rationale for an open label trial

The most important departure from the usual methodology of a randomized controlled trial will be the absence of blinding. This is justified by the primary objective to compare the effectiveness of the two regimens which will primarily be determined by the completion rate of therapy. The 9INH regimen has efficacy of approximately 90%[140] if taken fully - making it virtually impossible to demonstrate superior efficacy with 4RIF. However in routine practice fewer than 50% complete INH therapy [70,93,96-102], reducing effectiveness to less than 50%. On the other hand, in Phases 1[131] and 2[148], and in observational studies [123,124] treatment completion rates with 4RIF have been 20-30% higher than with 9INH – similar to trials comparing different durations of the same drugs, in which shorter duration consistently resulted in superior completion [65,159,160]. The fundamental rationale for this proposal and the basis for the sample size calculations is that the shorter duration makes improved effectiveness plausible, and detectable. To conduct a fully double blind study, patients assigned 4RIF would take an additional 5 months of similarly coloured and shaped placebo. This would eliminate the most important advantage of 4RIF, and under-estimate its effectiveness. As well, RIF produces reddish discoloration of the urine, so patients assigned to 9INH would take Iron oxide capsules for 9 months. This would render the interventions even less like routine practice, likely reduce compliance even further, and potentially reduce the effectiveness of both regimens to ethically unacceptable levels. Finally, the proposed design is consistent with all other non-placebo controlled trials of LTBI regimens of unequal length – which were all open label, and used microbiologically confirmed active TB as the primary end-point[61,66,105,117,161 65,115,115,136], as proposed here. The only published double blinded trials of LTBI therapy used placebo regimens of equal length[62-64 104 33,68,162]. However, given the consistent benefit of INH in these trials a placebo controlled trial would not be considered ethical.

2.1.2 Rationale for the duration of follow-up

The total duration of follow-up will be 28 months. This offers the best trade-off between the greater potential losses and higher costs associated with longer follow-up, and maximizing detection of incident cases of active TB, since disease risk is highest in the first 2-3 years after tuberculin conversion[42,43], detection of contacts[62], diagnosis of inactive TB [65], or following migration from high to low incidence countries [163 164]. Post-treatment follow-up will be unequal between subjects who complete therapy – 24 months after 4RIF, compared to 19 months after 9INH. This is justified because: (i) We are most concerned about therapeutic failure with 4RIF, given its unknown efficacy. Hence longer follow-up after therapy will enhance our chances to detect failure in compliant subjects – an outcome of key interest. (ii) On the other hand, given the well documented high efficacy of 9INH, therapeutic failure is unlikely in subjects who complete 9INH, so almost all events of active TB will occur in those who drop-out of INH therapy. Since most drop-outs occur early in therapy [148], length of follow-up after stopping treatment will actually be similar in those actually at risk to develop active TB; (iii) If anything, this approach will favour 9INH slightly, because patients who completed 4RIF will have 5 more months to be re-infected - plausible in high-incidence settings.

2.2 INTERVENTIONS

The standard therapy will be daily self-administered INH, 5 mg/kg/day (max=300mg/day) for 9 months (9INH). Dosage will be adjusted if weight is less than 42 kg at 200mg/day. As currently recommended [107,138,158] vitamin B6 (pyridoxine) will be given with INH only to patients with risk factors for neuropathy - malnutrition, alcoholism, diabetes, or renal insufficiency or HIV positive. The experimental arm will be daily self-administered RIF, 10 mg/kg/day (max=600mg/day) for 4 months (4RIF). Dosage will be adjusted if weight is 36-49 kg at 450 mg/day or at 300 mg/day for weight of 35 kg and less[107,138,165].

2.2.1 Rationale for the interventions

We have selected 9INH as the standard regimen because: (i) in current guidelines this is the preferred regimen for LTBI[107,138,158]; (ii) It is the current standard of practice in North America [97], used in 84% of patients treated for LTBI [57]; and, (iii) INH has consistently had

significantly better efficacy than placebo in all trials with study populations similar to the populations we plan to study [62, 141, 142]; (iv) in systematic reviews [62], and meta-analyses [141, 142] 9INH has efficacy of as much as 90% [140] if taken properly. We have selected 4RIF as the alternative regimen because this is one of three alternates recommended by the CTS and ATS [107, 138]. Of the two others, 6 months INH had efficacy of 40-69% in several trials [33, 61, 65, 68, 104, 128], and the 2RIF-PZA regimen has unacceptable hepatotoxicity [109, 112, 117, 145]. In our earlier trials and observational studies, 4RIF has had lower toxicity [123, 124, 148], lower cost [147]and better completion [123, 124, 131] than 9INH. There is evidence to suggest the efficacy of 4RIF is as high as that of 9INH, but efficacy has not been adequately assessed.

2.3 PROCEDURES OF RECRUITMENT, REGISTRATION, AND RANDOMIZATION

2.3.1 Recruitment:

Patients prescribed 9INH therapy will be asked, by their TB care provider, for permission to be contacted by study personnel. Those that agree will be approached to verify eligibility, and obtain signed informed consent. (See Research module Appendix 1 – informed consent used in Phase 2)

2.3.2 Registration

Once subjects have provided informed consent, research staff will access the registration and randomization website via the Internet. This computer based program will be available 24 hours/day and 365 days/year.

2.3.3 Randomization

We propose central, web-based randomization by a computer generated random number producing algorithm, in blocks of varying length (2 – 8 subjects), stratified by centre. Patients who are household contacts of active cases will be allocated to the same regimen as the first member of that household. Because study personnel may recruit more contacts to one arm if they believe it superior, all contacts in one household must be recruited and randomized at once. If additional household members are enrolled at a later date, they will be randomized separately.

2.3.4 Rationale for stratification by centre

The characteristics of the patient populations, their risk of TB, as well as physicians' usual practice – in starting LTBI therapy, or stopping it because of patient intolerance, will vary considerably by centre. Randomization stratified by site should ensure that these and other potential sources of bias by centre are balanced. As well this will ensure that balance is maintained if one or more centres stop enrolling [166], as occurred in Phase 2.

2.3.5 Maintaining Confidentiality

The Web-based registration system is non-nominal. At the time of registration, all patients are assigned a unique study ID number. This will be used to label forms, clinical data, and all case report forms. All patient information sent to the data coordinating centre, or reviewed by independent panels, will contain only the study ID. All contact information including names, address, telephone numbers, other contact persons, and full date of birth will be stored at each site, in a secure location, and safeguarded by the site PI. In Canada, lists of participants' names will be forwarded by registered courier to Provincial health authorities to assess if they develop active TB, following procedures approved by provincial privacy commissions. At the international sites the patients' names will be cross-checked against reported TB cases at State, or National level, using appropriate safeguards for patient confidentiality. Protocols will be written for protection of confidentiality; these will be a focus of training and ongoing monitoring of all sites.

2.4 PROTECTION AGAINST BIAS

2.4.1 Preventing bias in ascertainment of the primary outcome of active TB

Given the absence of blinding, it is important to control for potential bias in ascertaining the primary study outcome. To reduce bias in finding or diagnosing cases, the questionnaire used for follow-up, and the clinical evaluation including diagnostic procedures for active TB will be standardized. The diagnostic process may vary if subjects seek care from non-study providers, but this should not be biased by the LTBI regimen. Each member of the clinical panel will review all available clinical evidence including X-rays, pathology, and microbiology reports, while blinded to subjects' identities, LTBI therapy, and opinions of the other panel members. Each will independently determine if the definition of confirmed active TB is met – ie positive cultures or nucleic acid amplification test for *M. Tuberculosis*, or positive biopsy (see Section 2.8.1 for details). These objective microbiological criteria should not be influenced by patient, nor provider bias, and are the standard outcome for all LTBI trials [33, 61, 65, 68, 104, 128].

2.4.2 Preventing bias in ascertaining secondary outcomes

The secondary outcome of probable TB, defined as compatible clinical and radiographic features will be judged by the same 3-member clinical review panel, using the same approach as above. All possible Grade 3 or 4 SAE will be investigated using standardized protocols, and graded according to ATS guidelines for hepato-toxicity [155], or the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html) for all others. Grading, and attribution of cause, will be performed by three independent reviewers blinded to the identity of patients, study drug, and opinions of the other reviewers. We will ascertain costs based on visits, tests and other health services – all of which are quite objective. To further minimize bias, the laboratory technicians who perform

cultures and drug sensitivity testing, and the cost-effectiveness analyst will be blinded to study regimen.

2.5 INCLUSION AND EXCLUSION CRITERIA

2.5.1 Inclusion criteria

Adults (age ≥ 18) with documented positive TST as defined below and prescribed 9INH for LTBI, following authoritative recommendations [107, 138]:

1. HIV positive (TST ≥5 mm).
2. Close contact: ≥4 hours contact per week, for ≥ 1 week with person with active pulmonary TB. (TST ≥5 mm)
3. Apical/upper lobe fibronodular disease with area >2cm^2 (shown in RCT Quick Guide) (TST ≥5 mm.)
4. Documented tuberculin conversion within two years. (Increase ≥6mm, with subsequent TST ≥10 mm).
5. Diabetes, renal failure, or immuno-compromised from medical condition or therapy (TST ≥10 mm).
6. Casual contact: contact of <4 hours/week, with a person with smear positive pulmonary TB. (TST ≥10 mm).
7. Tuberculin conversion within 2-5 years. (Increase of 6mm or more with subsequent TST ≥10 mm.)
8. Have **two** of the following five factors if TST = 10-14mm, or **one** factor if TST ≥15mm:
   a. Arrival in Canada, Australia, or Saudi Arabia in the past 2 years from countries with WHO estimated incidence greater than 100 per 100,000 (these are listed in the RCT Procedure guide - Appendix 5)
   b. Less than 90% ideal body weight, calculated using the body mass index (BMI, see RCT Procedure guide);
   c. Any abnormality on chest x-ray compatible with past-TB infection e.g. calcified granuloma, or hilar lymph nodes, costo-phrenic angle blunting - other than fibronodular disease above.
   d. Cigarette smoking (at least a half pack per day) currently.

In the low and middle-income countries, LTBI therapy will usually be offered only to patients in categories 1& 2[158], because of resource limitations, resulting in substantial differences in risk of active TB among untreated patients at the different sites. Balanced allocation of risk groups to the two study arms will be maintained by site-stratified randomization.

2.5.2 Exclusion criteria

1. Patients who were contacts of TB cases known to be resistant to INH, RIF, or both (i.e. MDR)[107, 138, 127].
2. Known HIV-infected individuals on anti-retroviral agents whose efficacy would be substantially reduced by Rifampin, unless therapy can safely be changed to agents not affected by Rifampin (listed in RCT quick guide - Appendix 5).
3. Pregnant women - Rifampin and INH are considered safe in pregnancy [138, 167], but therapy is usually deferred until 2-3 months post-partum to avoid fetal risk and the potential for increased hepato-toxicity immediately post partum [168].
4. Patient on any medication with clinically important drug interactions with INH or RIF, which their physician believes would make either arm contra-indicated. An updated list of clinically important drug interactions is in the RCT Quick Guide (Appendix 5). This includes women taking hormonal contraceptives who will not take alternative contraception.
5. History of allergy/hypersensitivity to Isoniazid or to Rifampin, Rifabutin or Rifapentine.
6. Active TB. Patients initially suspected to have active TB can be randomized once this has been excluded.
7. Persons who have already started LTBI therapy.

2.5.3 Rationale for exclusion criteria

We are interested in the real world application of Rifampin, and so will include persons potentially at risk for non-completion or for adverse events. To obtain a realistic estimate of safety and tolerability, no patient will be excluded on the basis of age nor history of TB therapy, liver disease, alcohol use, or other medication use (except as specified above). If the treating physician prescribed LTBI therapy, they must have concluded the benefits of therapy outweighed the risks. Similarly, to obtain a realistic estimate of effectiveness, patients at risk of non-completion (homeless, alcoholic, and drug use) will be included. An additional reason for these inclusion criteria is to enhance comparability with earlier trials [169] - which involved subjects who were older[33, 65, 161], HIV infected[68, 104], or had other co-morbidities[62].

2.6 DURATION OF TREATMENT: (see section 2.2 – Interventions)

2.7 FOLLOW-UP AND DATA GATHERING

2.7.1 Initial evaluation

The initial evaluation, completed by study personnel, will include demographic information, past medical history, reasons for LTBI therapy, tuberculin reactions, chest X-ray findings, other investigations, and HIV status if known. In contrast to patients with active TB, HIV testing of LTBI candidates is not considered essential for patient care [107, 138, 158]. Hence HIV testing will be offered to all subjects, with appropriate counselling, although it will not be a required test for inclusion in the study. The need for other investigations is at the discretion of the provider, except for subjects with chest X-ray abnormalities with lesions ≥ 2cm^2, or close contacts with symptoms or X-ray abnormalities, in whom sputum specimens must be sent for AFB smear and culture [107, 138]. If investigations to exclude active TB are performed, patients can not be enrolled until results are available. We have developed a draft algorithm to identify and investigate subjects

with possible active TB (RCT Appendix 3). This will be evaluated and validated to facilitate the use of LTBI therapy in resource-limited settings after the trial is completed.

2.7.2 Follow-up during treatment

We wish to ascertain treatment effectiveness under routine programme conditions. Therefore, follow up will be in line with standard practice, and conducted by the initial treating physicians and TB clinic staff, meaning that visits will be monthly for the first 4 months, then every 8 weeks thereafter for 9INH. Blood count (CBC), and liver transaminases will be checked pre-treatment and at the first follow-up visit. Patients will be encouraged to call, or see their TB therapy provider or TB clinic staff, if they develop any new symptoms. Concomitant treatment is a potential problem with un-blinded therapy [166] but given the specificity of anti-TB drugs it is highly unlikely that any such therapy would be given, unless active TB was diagnosed. To monitor drug interaction, for medications whose drug levels can be monitored, these will be measured at 0, 2, 4 and 8 weeks, along with any related dosage changes. For anti-coagulants, anti-diabetics, anti-hypertensives or lipid lowering agents the clinical end-points will be monitored - at 0, 2, 4 and 8 weeks of therapy, plus related dosage changes.

2.7.3 Post treatment follow-up

After treatment is finished (or discontinued) post-treatment follow-up by telephone, or visit – at the subject's home, or at the health facility will begin. Follow-up will be every 3 months until 28 months post randomization on the date corresponding to 3 monthly intervals after randomization. Follow-up frequency is because of experience gained with Phase 2 participants – frequent contacts are needed, even if brief, to enhance retention, because so many move, change telephones, or lose interest. Our objective is to achieve a drop-out rate less than 10%.

2.7.4 Maintaining high quality data:

2.7.4.1 Training: At the start, we will hold a two day training course for all investigators and research coordinators (see Appendix 7- Time-Table) to review eligibility criteria, ethics, consent procedures, protection of confidentiality, registration, randomization, reporting of serious adverse events, and follow-up procedures during and after treatment. Particular attention will be paid to procedures to minimize drop-outs, investigation and management of SAE during therapy, and ascertainment of active TB. Staff at new sites will receive additional intensive training at the study coordinating centre

2.7.4.2 Supervision: The PI will visit all new sites within three months after beginning randomization, and all sites annually. The study coordinator will visit new sites at the time of trial initiation, all sites every three months in year 1 and every six months thereafter. We will verify that patients are approached, informed, and provide consent correctly. We will ascertain that documentation of IRB correspondence and consents are complete, confidentiality is protected, and that data entered in databases, or using the Web-based programmes, corresponds to source documents. Verbal feedback will be provided to staff immediately, and written reports will be reviewed with the site investigators and staff, and the principal investigator.

2.7.4.3 External Audit: For all Canadian sites, we plan two independent audits – the first 6-9 months after starting enrolment, and the second 18 months later. Both will be performed by a professional clinical research associate experienced in auditing pharmaceutical sponsored trials. For budgetary reasons, the professional auditor will make only one visit to each international site - one year after beginning randomization. The PI will make the second audit visit 12 months later.

2.7.4.4 Electronic databases - web based and local: The web based initial registration incorporates all information from the initial case report form. All SAE and active TB will also be reported using non-nominal web-based forms. To collect all other treatment phase, and post-treatment follow-up information, site staff will use local databases. The data will then be transferred –in non-nominal form- to the coordinating centre. All web-based forms and randomization software, as well as local databases were developed in Phase 2, except for reporting the primary outcome of active TB - which will be added.

2.8 THE PRIMARY AND SECONDARY OUTCOMES:

2.8.1 Definition of the primary outcome

Confirmed active TB during 28 months after randomization will be defined as a positive culture for *M. tuberculosis*, positive Nucleic acid amplification test for M TB complex, or caseating granulomas in a biopsy from any site. Positive AFB smears will be considered false positive if cultures are negative, but will be considered confirmatory, if cultures failed (for example if contamination or other technical problem occurs).

2.8.2 Definition of the secondary outcome of probable active TB

Probable active TB during 28 months after randomization will be defined as a compatible abnormal chest X-ray plus clinical symptoms, which improve following treatment for active TB, as judged on blinded review by a majority of the independent clinical review panel members.

2.8.3 Other secondary outcomes

See below for compliance, serious adverse events, costs, and drug resistance.

2.9 MEASURING THE PRIMARY AND SECONDARY OUTCOMES

2.9.1 The primary outcome of active TB

Our primary method to detect active TB will be active follow-up. Participants will be instructed to contact study personnel if symptoms suggestive of active TB arise – during, or after completion of therapy. During LTBI therapy, subjects will be questioned at each follow-up visit for symptoms suggestive of active TB. After therapy has been completed they will be contacted every three months by study personnel until 28 months after randomization. This will be done primarily by telephone in Canadian and Australian sites, by direct patient visits in Brazil and Africa, and both methods in Korea and Saudi Arabia. At each contact, standard questions (listed in RCT Procedures – Appendix 5) will be asked about current symptoms, and if they were diagnosed with TB since last contacted. Any patients with symptoms suggestive of active TB will be evaluated promptly by study personnel following a standardized protocol, including X-rays, sputum AFB smears and cultures. If subjects are diagnosed with active TB elsewhere, information will be collected regarding date of diagnosis, date and type of treatment, treating physician, and health facility. Permission will be sought to obtain clinical, laboratory, and treatment information, and copies of relevant X-rays from the treating physician.

2.9.1.2 Verifying the diagnosis of active TB: An independent 3-member clinical panel will review X-rays, and all clinical and lab information. All members are Montreal-based chest specialists with more than 20 years experience, including many patients with active and latent TB (see Research Module Appendix 1); none are co-investigators. Panel members will independently diagnose each case as confirmed, probable, or unlikely TB, as defined above, blinded to patient identity, LTBI regimen, and opinion of treating physician or other panel members. Differences will be resolved by consensus.

2.9.1.3 Assessing completeness of ascertainment of active TB: To verify the completeness of ascertainment of active TB with our proposed method of follow-up, we will send a list of all randomized subjects to public health officials responsible for the TB registry in each jurisdiction. They will match subjects names with nominal registries held at provincial, state, or national levels. This is included in the consents for Phases 2, and 3 (Research Module Appendix 1). This will allow us to ascertain any under-estimate of the primary outcome, although patients with clinically diagnosed TB, and those who developed TB after leaving the province/state where enrolled may be missed. We will use the same verification procedure for the international sites. In Canada, provincial registries are almost 100% complete for microbiologically confirmed cases, but the completeness, accuracy and comparability of the TB registries at the international sites are unknown. However any possible differences in detection between sites should not bias the comparison of regimens, given the stratified randomization by site.

For the Canadian sites we will ascertain outcomes of consenting participants who were lost to follow-up, through the provincial health administrative databases. We will forward the names of these subjects, to the provincial health authorities 28 months after randomization, to verify whether they have died (if so, date of death), or moved out of province (if so, when). This will enable us to ascertain outcomes of such subjects, and to examine potential bias due to these drop-outs.

2.9.2 The secondary outcome of compliance during treatment

Compliance with treatment is an important modifier of treatment effect, and must be measured to perform the planned efficacy analysis among the sub-groups who take therapy per protocol. In Phases 1 & 2 we used the Medication Event Monitoring System (MEMS), to record pill taking behaviour. However we plan to use pill counts as our primary method of assessing compliance because: (i) pill counting is inexpensive, and feasible for all programmes – hence results can be more easily reproduced elsewhere; (ii) in Phase 2 pill counts were highly concordant with the MEMS records (iii) the MEMS is very expensive, and so is unlikely to be adopted in practice – even in North America; (iv) in a previous trial, pill counting had excellent predictive value for risk reduction [65].

2.9.3 The secondary outcome of serious adverse events (SAE)

At each follow up visit patients will be questioned and examined for evidence of adverse events. Prior to beginning therapy and at the first follow-up visit, CBC, and liver transaminases (AST and ALT) will be tested. As in Phase 2, (see Section 2.19) site investigators will file an initial web-based SAE report if therapy is discontinued because of patients' symptoms or lab abnormalities. They will be investigated and managed following standardized protocols developed in Phase 2 (RCT Appendix 2), based on our published experience [170], recent reviews [154, 155] and authoritative guidelines [138, 155]. Adverse events will be graded as suggested in guidelines by the American Thoracic Society for hepato-toxicity [155], and the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html;). As in Phase 2 we will have an independent 3 member panel (Dr Rick O'Brien, Dr Mike Lauzardo, and Dr Wendy Cronin - letters in Research Module Appendix 1). This panel will review all possible SAE – defined as events that lead to permanent physician discontinuation of study drug, without knowledge of study drug, nor opinions of neither other panel members nor treating physician. They will judge type, severity, and probability of cause of the SAE. Differences will be resolved by consensus. Results of this blinded review by the independent panel will be considered the final diagnosis for type, severity, and relationship to the study drugs.

Detection of Grade 3-4 reactions may be greater than in routine practice, but this should occur equally for both arms, and enhance research subjects' protection. Unreported adverse events cannot be reviewed - leaving room for provider bias. This may occur with minor intolerance, (which will be termed patient non-compliance). However, if the provider stops therapy because of adverse events, this must be reported by investigators, and investigated as above. The anticipated number of participants in each arm will provide substantial power to detect relatively small differences in occurrence of SAE. This should provide the opportunity to detect less common SAE, such as hematologic complications, or ascertain if differences occur within subgroups (e.g. HIV infected). (See also Section 2.18).

2.9.4 The secondary outcome of drug resistance among cases of active TB

All positive mycobacterial cultures from subjects who develop active TB within 28 months post randomization will be sent to reference TB laboratories for identification and drug susceptibility testing. All international sites have access to reference TB laboratories, which participate in external quality control programmes with WHO supra-national reference laboratories.

2.10 MEASURING HEALTH SYSTEM COSTS

We will gather information on all health services (visits, consults, tests, drugs) used by participants' during treatment, and if they develop active TB, as an integral part of the local and web-based data-bases. Hence this information will be routinely gathered as part of the trial, as was done in Phase 2. Investigators at each site will provide site specific estimates for values (costs) for all these health services, using methods that we have used in earlier studies [54,171-173]. (Details in RCT Appendix 4).

2.11 SAMPLE SIZE REQUIREMENTS

Our primary objective is to test whether 4RIF has superior effectiveness compared to 9INH in reducing occurrence of confirmed active TB, based on a planned intention to treat analysis. The number required depends upon the estimated rate of active TB in treated, and untreated patients, and the expected completion rates with 9INH. The expected rate of TB is hard to predict, since we will enrol patients at a wide range of increased risk. In the international sites, where most patients will be contacts (risk over 2 years of 3% [62 174] up to 8-10% [63,175]), or HIV infected (**annual** risk 3-8% [20,21]), cumulative incidence if untreated (or non-compliant) should exceed 5%, but may be only 2% in untreated subjects in Canada. Hence we assume an average cumulative incidence of 3% among untreated. Based on the assumption that 9INH has 90% efficacy if completed [140], but only 50% completion rate [70,93,96-102], and 4IRF efficacy is 90% the required sample size, calculated using Poisson distribution, would be 2,898 participants with full follow-up in each group (see Table S1 below). If re-calculated using the binomial distribution (and *http://stat.ubc.ca/~rollin/stats/ssize/b2.html*) the sample size required would be 2% to 5% larger. Smaller differences in completion will reduce power, but higher event rates (that are certainly plausible) will substantially increase power.

Table S1: Sample size required to detect superior effectiveness of 4RIF compared to 9INH

(calculated using Poisson distribution [176], assuming alpha=0.05 and two-sided tests)

Expected cumulative incidence of TB over 28 months after randomization			N (per group) to detect difference, with power of:		
No therapy	**9INH** (Completion 55%, Effectiveness 49.5%)	**4RIF** (Completion 80%, Effectiveness 72%)	**60%**	**70%**	**80%**
3%	1.49%	0.84%	2,648	3,337	4,243
4%	1.98%	1.12%	2,013	2,537	3,226
5%	2.48%	1.4%	1,598	2,013	2,560
No therapy	**9INH** (Completion 50%, Effectiveness 45%)	**4RIF** (Completion 80%, Effectiveness 72%)	**60%**	**70%**	**80%**
3%	1.65%	0.84%	1,809	2,279	2,898
4%	2.20%	1.12%	1,357	1,709	2,173
5%	2.75%	1.40%	1,086	1,368	1,739

Members of the same household will be randomized to the same regimen. When cluster randomization is used, sample size must be adjusted accordingly because members of the same cluster are no longer independent observations [177]. Usually, the required sample size is inflated by a factor of $1+(m-1)*ICC$; where m is the average cluster size and ICC is the intra-cluster correlation coefficient which denotes how similar subjects from the same cluster are with respect to their risk of developing the outcome, compared to those in other clusters [178]. Given that the cluster sizes will vary (in Phase 2, 20% of subjects were household contacts, of whom 60% were single contacts, and 40%, or

8% of all participants, were in groups of 2-4 persons - with an average number of 2.5 contacts), we adjusted for this via the method described in [178]. This calculates the design effect as: $1+[(cv^2+1)m-1]*ICC$ where ***cv*** is a ratio of the standard deviation of the cluster sizes, ***m*** and ***ICC*** as before. Even assuming an ICC as large as 0.1 (ICC is typically 0.05-0.12 for spouse pairs [179]), this only results in a design effect of 1.03 - meaning a 3% increase to 2985 per group. Allowing for 10% loss to follow-up the number must be increased to 3283 per arm. Patients will continue to be followed even if they stop therapy themselves (i.e. non-compliance), or their physician stops therapy (e.g. pregnancy or adverse events). Because 847 subjects, enrolled in Phase 2 of this trial, are still being followed for occurrence of active TB using the same methods as proposed here, the total of number of new participants required in this phase can be reduced to 5720.

We have assumed 4RIF efficacy of 90%, based on available evidence. As shown below, if 50% of the 2,898 randomized to each group complete therapy and 28 months follow-up, this would provide more than 90% power, to confirm **non-inferior** efficacy of 4RIF, if the **non-inferiority** margin was 25% - equivalent to a minimum efficacy of 4RIF of 65%. (In other words, we would declare 4RIF **non-inferior** to 9INH if the efficacy of 4RIF was not more than 25% worse than 9INH.) This efficacy has been considered sufficient for authoritative recommendations of 6INH [107, 138], which has had efficacy of 40-69% in trials [33, 61, 65, 68, 104, 128].

Table S2: Sample size to assess non-inferiority of 4RIF efficacy

(calculated using alpha=0.05 and one-sided test using methods suggested by Blackwelder [180])

Expected cumulative incidence of TB		Tolerated difference		Number (per group) required to provide power of	
Untreated	**9INH**	**Δ (25%)**	**Maximum Event rate with 4RIF**	**80%**	**90%**
3%	0.3%	0.75%	1.05%	658	911
4%	0.4%	1%	1.4%	493	683
5%	0.5%	1.25%	1.75%	394	546

2.12 PLANNED RECRUITMENT RATE: (see also Section 2.15)

At the Canadian sites, we anticipate 455 subjects enrolled annually, based on an expectation of similar enrolment at continuing sites, and the addition of Vancouver. The Vancouver site is the largest TB clinic in Canada with more than 800 persons treated for LTBI annually. Given the allocation of the same budget as for Montreal and with 33% more patients, it seems realistic to expect similar recruitment rates as in Montreal. We plan to double the capacity in Brazil by doubling staff and doubling the number of clinics at which patients will be enrolled. We anticipate little change in enrolment in Saudi Arabia. Two new sites will be added in West Africa; both have substantial experience and infrastructure for the conduct of randomized trials of active TB. We anticipate a rapid start-up with an average of 500 enrolments annually from both sites (letter from Dr. Lienhardt - Appendix 1). In Seoul, Korea four University hospitals and the very busy clinic at the Korean Institute of TB (KIT) will be added, under the direction of Dr Woojin Lew (Director of KIT - see letter in Appendix 1). Investigators at KIT were recently awarded a major grant for a 10 year longitudinal study of 3,000 close contacts. It is anticipated that at least 500 contacts with LTBI will be recruited annually at this site. In Australia and Saudi Arabia, the patient volumes are much lower, but addition of these two sites will be valuable because of the high quality TB programmes, and potentially greater generalizability of results to a broader range of settings. In addition both sites will cover all local costs – hence only travel costs for meetings, training, and supervision will be needed (see letters from Dr Marks, and Drs Al Jahdali and Memish - Appendix 1). Dr. Menzies will spend three months during years 1 and 2 in getting the study initiated at all these sites. (This plan is supported by his department Chair - see Dr Eidelman letter in Appendix 1). If all sites enrol a total of 1,950 participants annually (see section 2.15), we should complete enrolment within 3 years, although we have planned recruitment over 3.5 years. And, as in Phase 2, enrolment will be monitored closely, and corrective actions taken promptly - including addition of sub-sites, and, if necessary, shifting operating budgets from under-performing sites.

2.13 ANTICIPATED PROBLEMS WITH COMPLIANCE AND TREATMENT COMPLETION

As discussed in Section 2.3, we expect differences in treatment completion to be the major determinant of differences in effectiveness of the two regimens. Our sample size calculations are based on these expected differences in completion rates, so recruitment will not be further increased to account for non-completion of therapy. We do not anticipate a major problem with "drop-ins" [166] during treatment i.e. patients who change therapy and cross over from one arm to the other, as this was seen in less than 1% of subjects randomized in Phase 2. Our planned secondary analysis of efficacy will be based on treatment completion, and assessment of compliance based on pill counting. This measure correlated well with electronic monitoring in Phase 2, and with protective effect in prior trials [65].

2.14 LOSS TO FOLLOW-UP

Our ability to follow all participants successfully for 28 months after randomization will be crucial. Our objective is to have less than 10%

drop-outs; higher rates would be of concern, given the anticipated rate of the primary outcome of less than 3%. Loss to follow-up was 17% in Phase 2, although over 97% of subjects had some follow-up (see RCT Appendix 1: Table 7). Based on experience gained in Phase 2, we will ask for home, work, and cellular telephone numbers, plus email addresses of the study participants, at the time of their enrolment. We will also ask for four other contacts – close friends, or relatives living in the same city, or same country, or relatives remaining in their home country for recently arrived immigrants. This approach has been successful, in another ongoing trial of LTBI therapy at the Montreal Chest Institute, in keeping losses to less than 5% of those randomized, during 33 months of follow-up. The initial consent will include subjects' permission to verify their occurrence of active TB using local nominal TB reporting databases – for all sites, and provincial health administrative data-bases for Canadian sites. This will provide a mechanism (that we have used [181, 182]) to verify occurrence of active TB passively, and assess under-estimation of the study outcome, due to mortality or migration.

2.15 DESCRIPTION OF STUDY CENTRES, AND JUSTIFICATION FOR INTERNATIONAL SITES:

Selection of the international sites has been based on long-standing collaborative ties [172, 183-191, 192] between the site investigators and the PI. All involved countries have a substantial burden of TB, and are classified as having intermediate or high incidence [2]. Inclusion of these sites strengthens the study for several reasons: (i) TB is a global disease that disproportionately affects low and middle income countries, where it is a very high priority health problem. (ii) LTBI therapy is under-utilized in these countries because it is viewed as impractical – but could have important benefits if therapy was simplified. (iii) Conduct of the trial in these international settings may help to demonstrate the feasibility, and cost-effectiveness of this strategy in these settings. This may enhance uptake of the findings in countries with a substantial TB burden. (iv) The training and conduct of this trial will strengthen capacity for clinical research in each country - an important long-term benefit. (v) These sites enhance the feasibility, and cost-effectiveness of the present trial.

Participating Canadian and international sites in Phase 3. (*Four will be new sites. **Brazil will double clinic sites)

	Number of patients treated per year (from 2005-2007)		Eligible per year, (estimated for new sites)	Annual enrolment	
	Active TB	LTBI		Phase 2	Projected
Montreal Chest	60-70	550-650	200	140	150
Saskatoon	80-90	300	50	25	30
Toronto	60-70	250-300	50	25	50
Edmonton	70	450	100	60	75
Vancouver*	120	800	250-300	--	150
Rio de Janeiro, Brazil**	800	>1,000	>500	180	350
Riyadh, Saudi Arabia	100	300	150	70	70
Korea*	2560	na	2500	na	500
Australia*	300	123	120	na	75
Benin*	>1,000	na	500	na	250
Guinee*	>1,000	na	500	na	250
TOTAL	>2,000	> 4,000	> 1,600	500	1950

2.16 PRIMARY DATA ANALYSIS

The primary study outcome will be the occurrence of microbiologically or histologically confirmed active TB, confirmed by the majority of the 3-member independent clinical review panel. The primary analysis, comparing the rate of occurrence of active TB per patient-year will be performed with the use of an unadjusted Poisson marginal model fitted using generalized estimating equations (GEE) to allow us to take clustering by household into account [193, 194]. We will assume an exchangeable correlation structure. The log of follow up time will be used as an offset in the regression model, which will allow us to account for differing lengths of follow up time [194] If clustering is significant we will calculate a rate ratio from the GEE Poisson regression. But if the effect of clustering is negligible, as anticipated, the proportion of subjects randomized to both treatment groups developing confirmed active TB, and the associated 2-sided 95% confidence interval for the difference, will be estimated, using an incidence density method, expressed as TB events per 1000 person years of follow-up. This will allow us to include information from subjects who are followed for some time before being lost to follow-up.

2.16.1 Justification

Our planned primary analysis will be of study groups as randomized, i.e. an intention to treat analysis. This is recommended for superiority studies [166] because it provides a more conservative estimate of effect, since patients who did not take treatment per protocol are less likely to gain benefit. Hence differences between two regimens are attenuated making it more difficult to detect superiority.

2.17 FREQUENCY OF INTERIM ANALYSIS AND STOPPING RULES

2.17.1 Primary outcomes

Only one interim analysis of the primary outcome will be performed, one year after 33% of patients have been randomized. Further interim analyses, such as one year after 67% of subjects have been randomized, will fall too close to the end of randomization, to have any meaningful impact. We wish to avoid falsely concluding that one regimen has significantly superior effectiveness with this interim analysis – a well known risk [195,196]. Hence we will use a threshold of a p value <.001[196], before concluding that 4RIF is significantly **inferior** to 9INH , and stopping the trial early.

2.17.2 Serious adverse events (SAE)

Four months after randomization of 25%, 50%, and 75% of subjects, interim analyses will be performed of SAE. The DSMB will consider stopping the trial early if SAE rates are significantly ***higher*** with 4RIF. To balance the risk of unnecessarily stopping the study with interim analyses [196], with the need to ensure patient safety, we will use the method of Pocock [197], or an interim stopping level (p value) of 0.018 for each analysis.

2.18 PLANNED SUB-GROUP AND SECONDARY ANALYSES:

2.18.1 Active TB in subjects who complete treatment (efficacy)

Non-completion of therapy is obviously not a random event, and may be associated with characteristics that are associated with risk of disease[166]. Therefore we will compare the characteristics of compliant and noncompliant subjects in each group, and use logistic regression to estimate efficacy, adjusted for important covariates.

2.18.2 Confirmed and probable active TB

In secondary analysis we will combine these two outcomes, and use the same methods to compare rates of TB with both regimens, as described above for the primary analysis.

2.18.3 SAE

This outcome will be defined as the occurrence of Grade 3 or 4 SAE, diagnosed by the majority of the independent reviewers. Differences in all SAE, and by type of SAE between the two groups will be tested with Chi-squared or Fisher's exact tests. Sub-group analyses will compare rates of SAE by age, sex and HIV status. Drug interactions with 4RIF are common, and of particular interest since this will be the largest trial with mono-Rifampin therapy. All analysis of SAE and drug interactions will be presented as exploratory, with appropriate caution [166,196]. However in view of the very limited published experience with 4RIF, any information about adverse effects, including drug interactions, would be very useful for clinicians.

2.18.4 Drug resistance among cases of active TB after randomization

The occurrence of drug resistant TB after LTBI therapy may result from selection of drug resistant mutants during therapy (acquired resistance), or because they were infected with drug resistant organisms (primary resistance). All patients with positive cultures for M Tuberculosis within 28 months after randomization will have drug sensitivity testing. Adequate facilities are available in all sites. If drug resistant tuberculosis is found, patients will be placed on appropriate therapy and followed by the site investigators in collaboration with the local TB programs. We will compare the prevalence of resistance to INH and RIF among study participants who develop active TB. The prevalence of underlying (or primary) INH resistance in the community will be assumed as the prevalence in those who took 4RIF, and of RIF resistance in those on 9INH. Acquired RIF resistance will be taken as the difference in prevalence of RIF resistance between subjects on 4RIF and 9INH. Power to detect significant differences will be limited, unless drug resistance is very common, but descriptive information on any excess resistance would still be useful.

2.18.5 Cost-effectiveness of the two regimens

We will have detailed information on all health system activities – including scheduled visits, therapy and tests as part of routine follow-up, unscheduled visits, tests and therapy for adverse events, and diagnostic and therapeutic activities for the cases of active TB that develop. These will be valuated using local costs for all such activities (for details see RCT Appendix 4). We will also have measured incidence of active TB among subjects randomized to the two LTBI regimens. If one arm is cheaper and associated with fewer TB cases then it will be clearly preferable from a cost-effectiveness standpoint. If one arm is more expensive but associated with fewer TB cases then we will calculate the incremental cost per additional TB case prevented by the more effective (but more expensive) regimen. In primary analysis we will use average Canadian costs to valuate all health care activities, but in secondary analyses will perform the same analyses using costs from each site. This will be of particular interest in lower-income countries, where LTBI therapy is considered a low priority [158,199]. In sensitivity analyses we will vary the costs for active TB, given that the cases detected in this study will likely be at an early stage, so may underestimate average costs.

We will also vary the costs of rifampin, which is extraordinarily expensive in Canada, compared to international prices[126].

2.19 RESULTS FROM PHASE 1 AND PHASE 2

Six years ago, we initiated a series of studies to evaluate 4RIF as therapy for LTBI. We felt the first essential requirement for 4RIF was that compliance and treatment completion had to be better than with 9INH. Hence these were primary end-points of the first study (Phase 1). Of 116 patients randomized equally to 4RIF or 9INH, 91% completed 4RIF with good compliance compared to 70% of those randomized to 9INH ($p<0.001$)[131 200] (See Research Module Appendix: Reprints).

The experience in 2000-2001 with 2RIF-PZA mandated a careful assessment of safety, conducted in Phase 2 - a multi-centre study primarily to compare serious adverse events (SAE) with 4RIF and 9INH. Consenting patients were randomized in equal numbers to 4RIF or 9INH using a web-based patient registration and randomization developed by Dr. Rousseau of the University of Sherbrooke. *To view the registration, randomization, and SAE reporting web-site, use Internet explorer (at least 6.0) to go to:* http://tbera-demo.crc.chus.qc.ca. *Username:* **dmenzies**, *password:* **review**; *domain:* **rsr** *(until Feb 1 2009). Enter these once when prompted, and then enter the same username and password a second time, when prompted again. This gives administrator rights to view a demonstration database, with fictitious patients.* Randomization was stratified by site in blocks of variable size. Investigators performed a standardized evaluation for each possible SAE. If the study drug was permanently discontinued, all clinical, lab, and follow-up information was reviewed independently by a three member panel, blinded to the study drug, to judge the type, severity and likely relationship to the study drug (Appendix 4).

We experienced more difficulties recruiting patients than anticipated at several Canadian sites. In the first year we intensified training and supervision, and opened a new sub-site. When this was not enough, investigator and CIHR approval was sought to shift budget allocations from low enrolling sites to start two new international sites – in Brazil and Saudi Arabia, ***within the originally awarded total budget***. Enrolment at all sites subsequently increased to average 40 patients per month. We had planned a sample size of 549 per arm to provide 80% power (2 sided test) to demonstrate a significant difference in SAE rate if the true rate with 9INH was 4% and 4RIF was 9%, or was 5% with 9INH and 2% with 4RIF. After 25%, 50% and 75% of planned enrolment, the DSMB reviewed interim analyses – still blinded to the study drugs. In January 2007, the 3rd interim analysis revealed that SAE were significantly fewer in one arm. The DSMB asked to be unblinded, and when they learned that 4RIF had the lower rate of SAE, they recommended discontinuation of enrolment. Of 420 subjects randomized to 4RIF, 7 developed Grade 3-4 adverse events attributed to study therapy by the independent panel, compared to 17 of the 427 on 9INH (Risk difference (4RIF-9INH): -2.3%; [95% Confidence interval: -0.1% to -5%] p=.04). Grade 3-4 hepatitis occurred in 3 taking 4RIF, compared to 16 who started 9INH (-3.1%; [-1% to -5%], p=.003). Grade 1 or 2 adverse events attributed to study drugs were similar in the two arms. Asymptomatic reduction in platelet count and white blood count were significantly more frequent during treatment with 4RIF. Completion rates were 78% with 4RIF and 60% with 9INH (Risk difference: 18% [12% to 24%], p<.001) [148]. Average health system costs were significantly lower with 4RIF. Incremental cost effectiveness analysis revealed that 4RIF would be cost saving and prevent more cases if efficacy was at least 75%, and would be cost saving if efficacy is more than 65% [147].

We believe that a study to assess effectiveness of 4RIF is now justified. This regimen has been demonstrated to have better completion, lower costs, and is safer than 9INH, particularly for hepatotoxicity - the most important and potentially lethal adverse event of INH therapy (and of 2RIF-PZA). In Phase 2, 847 subjects were enrolled, randomized, treated and followed to detect active TB - using the methods proposed here, making the present proposal more cost-effective.

SECTION 3 TRIAL MANAGEMENT

3.1 DAY TO DAY RUNNING (See also RCT Appendix 7 - Time-Table)

This is described in Sections 2.3 (recruitment), 2.7 (follow-up/data gathering), and 2.8-2.9 (measuring outcomes).

3.2 ROLE OF APPLICANTS

Dr Menzies will chair the trial steering committee, supervise the central coordinating staff, liaison with site PI's, and take primary responsibility for overall data analysis and report writing. Each site PI will supervise all aspects of site trial management (ethics, recruitment, and follow-up), and site-specific staff. To take advantage of members' expertise, added specific responsibilities will be assigned as described in detail in RCT Appendix 6.

3.3 TRIAL STEERING COMMITTEE, DSMB, AND CLINICAL REVIEW PANEL

The trial steering committee will consist of the PI, site investigators, and study manager (Mme Dion). They will meet by telephone conference every 3 months in the first year, and then every 6 months, to review recruitment, randomization, operational issues, and DSMB reports. This committee will decide on early termination (if recommended by the DSMB), major protocol and/or consent modifications, and budget re-allocations. The membership, responsibilities, and functioning of the DSMB are described in Section 2.9.3, and of the clinical review panel in Section 2.9.1.

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FINAL STUDY PROTOCOL (VERSION 6: July 2011)

A randomized clinical trial of 4 months Rifampin vs. 9 months Isoniazid for latent TB infection. – Phase 3 effectiveness

SECTION 1 THE NEED FOR A TRIAL

1.1. WHAT IS THE PROBLEM BEING ADDRESSED?

1.1.1 Importance of tuberculosis (why study TB?)

1.1.1.1 Globally: As a disease, tuberculosis (TB) is unique in that it is wholly preventable and treatable, yet on a global scale, incidence and mortality continue to rise [1]. The World Health Organization (WHO) has estimated that there are between 8 and 9 million new cases each year [2], that 200 million persons alive today will develop active TB during their lifetime [2], and that 30 million will die from TB over the next decade [2] - equivalent to the population of Canada. TB remains the world's most important infectious cause of morbidity and mortality among adults, yet remarkably, many consider this disease to be of little current significance or importance [3]. In many industrialized countries, after decades of decline, incidence of tuberculosis increased in the 1980's. In most, with greater investment in TB control, rates have since levelled off or declined somewhat [4,5] although in some, such as Britain [6], incidence continues to increase.

1.1.1.2 TB in Canada (why study TB in Canada?): Incidence of active TB in Canada declined steadily from the beginning of the 20th century until the mid 1980's. Since then, the number of new cases reported each year has remained largely unchanged. In 2006, overall incidence was 5 new active cases per 100,000 population [7], but rates were substantially higher in certain populations and regions, because TB remains a disease of poor and marginalised populations [8-10]. Incidence among aboriginals ranges from 25 to 50 per 100,000 compared to less than 3 per 100,000 among non-aboriginal Canadian-born [7]. Rates among the foreign-born are three times higher than the national average [7]; 90% [11-14] of cases among foreign-born arise because of reactivation of dormant TB infection acquired before immigration, in their countries of origin [15-17].

1.1.1.3. Tuberculosis in children: TB in children has been considered a low priority because their burden of disease was believed to be low, and children typically have non contagious forms of TB. However, WHO has estimated that children account for approximately 900,000 cases, or 11% of all cases globally. The burden is greatest in high incidence settings. For example in South Africa, 14% of all active cases occurred among children <12 years old, who had incidence of 400/100,000 - about half the rate in adults. The serious consequences of TB in children was demonstrated by an autopsy study in Zambia which found that TB rivalled pneumonia as a cause of death in young children.

1.1.2 Pathogenesis of tuberculosis (why treat latent TB infection?)

TB infection is transmitted by the airborne route from patients with active pulmonary TB disease. In more than 95% of individuals who acquire primary infection, there is no clinical illness and the TB bacilli enter a latent or dormant state; this may last only six months or lifelong. Latent TB infection (LTBI) causes no symptoms, and is not contagious. Usually the only detectable abnormality is a positive tuberculin skin test. The World Health Organization has estimated that close to **two billion people** have LTBI [2,3], of whom approximately 10% will reactivate over their lifetime. Therapy can reduce the likelihood of future active disease, but is inefficient, because the subgroup that will develop disease can not be distinguished from the majority who will not, although some have recognized risk factors (RCT Appendix 1: Table 1) [18-53].

1.1.2.1 Pathogenesis in children: The most important risk factor for development of active TB in children, and for greater morbidity and mortality following primary TB infection is younger age, as seen in Table 1 below. HIV infection is also a very important risk factor, while other causes of immune compromise are likely to increase risk of TB reactivation in children (as they do in adults), but are not well described in the published literature. Malnutrition is a third important risk factor , although the magnitude of risk is not well documented.

Table1: Risk of disease in children following primary TB infection (Adapted from Marais)

Age	Risk of disease following primary infection			
	Disseminated TB or TB meningitis	Pulmonary TB	No disease	Comments
<1 years	10 -20%	30-40%	50%	High rates of morbidity and mortality
1 – 2 years	2-5%	10-20%	75-80%	High rates of morbidity and mortality
2 – 5 years	0-5%	5%	95%	
5 – 10 years	<0-5%	2%	98%	Safe or "Golden years"
>10 years	<0.5%	10-20%	80-90%	Effusions or adult-type pulmonary disease

1.1.3 Importance of therapy of latent TB infection (how many are treated?)

In a recently completed survey, we found that more than $25 million is spent annually in Canada for the diagnosis and treatment of approximately 20,000 persons with latent TB infection in 2006[54]. One survey of 110 US health departments found that 127,996 persons initiated LTBI therapy between 2000-2002 [55]. Based on this, and a second survey of 37, 857 patients in 244 U.S. health departments, it has been estimated that 290,000- 433,000 persons are treated annually for LTBI in the US [56], of whom more than 80% receive Isoniazid daily for

9 months (9INH) [57]. This data and reports from other countries [58-60] indicate that LTBI therapy is a major component of TB control in many high income countries. In low and middle income countries the World Health Organization has recommended LTBI therapy for close contacts, especially children, of patients with smear positive pulmonary TB, and is promoting expansion of use of INH for HIV infected persons with LTBI.

Current therapy of LTBI in children: Children are excellent candidates for LTBI therapy. As noted earlier their risk of disease is high, particularly under the age of 5, they tolerate most anti-TB agents well, and the benefits of LTBI therapy are long-lasting. INH for 6 to 9 months is the current standard for therapy of LTBI in children, recommended by the WHO, and other authoritative agencies. However, documented completion rates are less than 50% and severe adverse events, although rare, can occur. Problems that further reduce the impact of LTBI therapy in adults, such as physician non-compliance (fearing serious side-effects), and poor patient acceptance have not been well studied in children, but are likely to further reduce the impact of LTBI treatment. As concluded by several authoritative reviews, a major limitation of LTBI therapy in children is the extremely limited evidence base, with particularly few studies of alternative regimens - even regimens which have been studied quite extensively in adults.

1.1.4 Need for a trial of therapy of latent TB in children

Several recent authoritative reviews have pointed out that a major priority in paediatric TB is the development and assessment of alternative LTBI treatment regimens. These reviews have noted that almost all prior randomized trials of LTBI therapy excluded children, primarily because of the difficulties of bacteriologic confirmation of pediatric active TB, since 70-80% of active pediatric TB cases are diagnosed clinically. Hence the end-point of bacteriologically confirmed active TB can not be used in pediatric LTBI trials - as in LTBI trials in adults. These expert reviews concluded that the evidence of efficacy derived from trials in adults could be extrapolated to children, and that the primary end-points in paediatric LTBI trials should be tolerability/safety and acceptability.

1.2 THE PRINCIPAL RESEARCH QUESTIONS

Adult:

Hypothesis: Therapy of latent TB infection (LTBI) with four months of daily Rifampin (4RIF) will result in cumulative incidence of microbiologically confirmed active TB during 28 months following randomization, that is significantly lower than the cumulative incidence of active TB among participants randomized to nine months of daily isoniazid (9INH).

Primary objective: To compare the cumulative incidence during 28 months after randomization, of confirmed active tuberculosis (TB) among all persons randomized (effectiveness, using intention to treat analysis) to 4RIF and 9INH.

Secondary objectives:

(i) Compare the cumulative incidence of confirmed active TB among those who took at least 80% of doses of the LTBI treatment to which they were randomized, in less than 120% of the allowed time (i.e. ***efficacy***).
(ii) Compare the cumulative incidence of ***probable***, as well as confirmed active TB between patients randomized to the two regimens during 28 months following randomization.
(iii) Compare rates of ***Grades 3&4 adverse events*** during treatment between subjects randomized to the two regimens.
(iv) Compare ***health system costs, and cost-effectiveness*** of the two regimens, in the different sites.
(v) Describe occurrence of ***drug resistance*** (to INH or RIF) among subjects who develop confirmed active TB.

Children:

Hypothesis: Among children at high risk for development of active TB, intolerance/adverse events will not be worse (non-inferiority), among those randomized to 4RIF compared to those randomized to 9INH. In addition completion of LTBI therapy will be significantly greater (superiority), and subsequent rates of active TB will not be significantly higher (non-inferiority) in children taking 4RIF.

Primary Objective

1. To compare the rates of premature discontinuation of study therapy because of adverse events of all grades judged probably related to 4RIF or 9INH, by the majority of an independent panel of 3 reviewers, blinded to study drug.

Secondary Objectives

1. To compare the rates of study drug completion of all children randomized to 4RIF or 9INH. Completion will be defined as taking at least 80% of total planned doses within 23 weeks for 4RIF, or within 52 weeks for 9INH.
2. To compare the rates of clinically diagnosed active TB as judged by an independent panel of paediatricians, up to 16 months post randomization in children who complete study therapy per protocol (efficacy).
3. To describe the occurrence of drug resistant microbiologically confirmed active TB among children randomized to the two arms, during 16 months post randomization.

BIOMARKER COMPONENT

(Refer to Appendix 10 for more background information for investigators regarding the biological and scientific rationale, as well as details of laboratory methods):

Hypothesis:

Decreasing T-cell cytokine (IFN-γ and IP-10) responses to stimulation with TB specific antigens are biomarkers of successful therapy of LTBI in adult. Hence decreases in cytokine response will be greater in persons who complete LTBI therapy than those who do not complete, and more rapid in those who take RIF than those who take INH.

Primary Objectives

1. To compare the change in T-cell cytokine response to TB specific antigens from therapy initiation to four months later between compliant adults allocated to 4RIF vs. 9INH. Compliant adults are those taking at least 80% of the allocated study drug up to that time.
2. Among adult participants in this trial, to compare the change in T-cell cytokine response to TB specific antigens from initiation of therapy to 9 months later (i.e. after treatment completion), by level of compliance as estimated by the percentage of recommended doses taken of 9INH or 4RIF.

Secondary objective:

3. To create a specimen bank by storing plasma and serum for future studies of other potential biomarkers.

1.3 WHY IS A TRIAL NEEDED NOW?

1.3.1 History of preventive therapy ("the INH story") (see RCT Appendix 1 Tables 2&3) [61-73]

In the 1950's and 1960's, a number of large scale placebo-controlled randomized clinical trials of Isoniazid (INH) therapy for LTBI were conducted. In trials where high compliance was achieved, or in subgroup analysis of "completer-compliers" 12 months of INH resulted in risk reductions of 83-93%, relative to placebo [63,65,67]. About 50,000 subjects received INH, yet adverse events, including hepatitis were uncommon, and there were no deaths related to drug induced toxicity in these studies [61,62,64]. As a result of this evidence of efficacy and safety, in 1971 the American Thoracic Society (ATS) issued recommendations strongly encouraging INH therapy of LTBI [74]. The resultant widespread use of INH was quickly followed by the widespread occurrence of hepatitis - with fatalities[75 69 76], which, together with apparently contradictory risk benefit analyses [77-81], resulted in widespread doubts about the benefits, and revised recommendations for use of INH[82-84].

Publications in the past decade have reported much lower rates of hepatotoxicity [70,72,73,85] and mortality [70-72]., which may be due to better selection of candidates for therapy, or closer follow-up. Recent analyses have concluded that INH therapy will be of benefit and cost-effective for healthy infected persons without risk factors, and be very beneficial and cost-effective for infected persons at higher risk of disease [86,86,87,87-91]. However the overall effectiveness of INH remains low – because of physician under-prescription [92-96] (fearing serious side-effects), and poor patient compliance (because of the long duration) [70,93,96-102], and costly[94]– because of the close monitoring required to detect potential serious, even fatal, adverse events.

1.3.2 Problems with INH in children:

The major problem with INH is sub-optimal completion, related to the long duration for an asymptomatic condition. In addition severe hepatotoxicity, although uncommon, can occur, necessitating close monitoring, and reducing the appeal of this regimen for providers, patients, and their parents.

1.3.3 Alternates to INH for therapy of LTBI (RCT Appendix 1: Tables 4&5)

These problems of INH therapy have stimulated substantial interest in the evaluation of shorter regimens for LTBI.

1.3.3.1 *Rifampin and Pyrazinamide ("The RIF-PZA story" – history repeats itself):* Based on initial animal studies [103] and randomized trials among HIV infected [104-106] the regimen of 2 months daily Rifampin & Pyrazinamide (2RIF-PZA) was recommended in 2000 [107]. This was soon followed by reports of severe and fatal drug induced hepatitis [108-111]. In subsequent studies, serious adverse events, particularly hepatitis were significantly higher among patients given 2RIF-PZA than in patients given INH [58,112-115 116 55,112,115,117,118], despite close monitoring. Interestingly, default rates [113,115,117,119] and costs [113,117] of 2RIF-PZA were the same, or higher than 9INH in several studies. As a result this regimen has been almost totally abandoned [120].

1.3.3.2 - Rifampin alone: The only published randomized controlled trial with a mono-RIF regimen compared 3 months RIF, 6 months INH, 3 months INH-RIF and placebo. Interestingly, the 3RIF regimen had efficacy of 63%, which was superior to all other regimens, and no hepatotoxicity [33]. 6 months RIF was given to 157 high school contacts[121], and 49 homeless contacts [122] - of INH-resistant cases. In both series 6RIF was well tolerated, with no subsequent case of active TB. Further evidence of the high efficacy, despite much shorter therapy comes from experience with active TB. In trials with head to head comparisons, addition of RIF allowed the total duration of therapy to be halved [158,167]. Hence, implicit in our study hypothesis is that efficacy of 4RIF is 90% - the same as 9INH. Several observational studies of LTBI therapy provide further evidence of the advantages of 4RIF. In the first, of 1,379 patients on 4RIF, 1 (0.1%) developed hepatitis, and 987 (72%) completed therapy, compared to 12 (2%) with hepatitis and 405 (52%) completion among the 770 who started 9INH [123]. In the second, 261 subjects initiated 4RIF, of whom 210 (81%) completed therapy, and 8 (3%) developed SAE (no hepatitis), compared to 113 (53%) completing, and 13 (6%) with SAE (3 with hepatitis) of the 213 who started INH [124]. In a third study, of 749 given 4RIF, 76% completed and 9 (1.2%) developed SAE (3 = 0.4% with hepatotoxicity)[125]. The past problem of higher cost of RIF has been resolved by dramatic price reductions in the international market [126]. There has been no emergence of INH resistance following INH therapy of LTBI in

several large studies [62 33,65,127], nor emergence of Rifampin resistance following RIF therapy [33,122,123,128] except for one case-report of a patient who was very poorly compliant with therapy [129]. Rifampin containing regimens would also be more cost-effective than 9INH for LTBI treatment in immigrants from countries with high rates of INH resistance [130]. In summary, mono-RIF therapy can be effective for LTBI, may be more cost-effective[130], with better completion rates [131], and less hepatotoxicity [33,121-124].

1.3.3.3 INH and Rifampin (INH-RIF): In the Hong Kong study, the INH-RIF combination was the most toxic, and least effective of the three active regimens [33]. A large uncontrolled paediatric case series utilizing regimens of 6, then 4, then 3 months of daily INH-RIF, reported few cases of TB among those treated, although community rates were used for comparison[59]. A recent questionnaire survey reported that 3 of 344 paediatric household contacts treated with 3INH-RIF developed active TB – a rate of 8.7/1,000 [60] . This was 48% less than the rate of TB among similar subjects who received placebo in earlier trials [62]. In Uganda, 3 months daily INH-RIF-PZA was less effective than 6INH, with similar completion rates, and 4 times higher SAE [128]. In Saskatchewan twice weekly directly observed INH-RIF was well tolerated, with higher completion rates, and lower subsequent TB incidence than patients given 12 months INH [132]. In three recent trials, completion of 3-4 months of INH-RIF was better than 6INH, with similar adverse events and efficacy [133-135]

1.3.3.4 INH-Rifapentine (INH-RPT): RPT is a new Rifamycin with a half life five times longer than Rifampin that can be given once weekly. In a recent randomized trial, 2 of 206 (1%) TST positive household contacts receiving 3 months of directly observed once weekly RPT-INH developed Grade 3 or 4 hepatitis, compared to 20 of 193 (10%) of subjects who received 2RIF-PZA [136]. Active TB developed in 3 (1.5%) who received 3INH-RPT, compared to one (0.5%) of the 2RIF-PZA group. A large CDC-sponsored trial comparing 3INH-RPT and 9INH is nearing completion. However RPT is an "orphan drug" produced in limited quantities by the manufacturer, that has had poor results in treatment of active TB [137]. Its utility may be limited because of the need for direct observation - impractical for private providers, and increasing costs.

1.3.3.5 The risk of drug resistance from LTBI mono-therapy (INH or RIF)

Drug resistance has been reported among patients who have taken LTBI therapy. The most common reason is that the original latent infection was drug resistant - termed primary resistance. In this case the LTBI therapy is simply ineffective. A more serious concern is that the infecting organisms acquire drug resistance as a result of the LTBI therapy itself. This acquired resistance is very unlikely in true latent infection as the bacillary burden is very low. However if mono-therapy – with INH or RIF – is given to someone with unsuspected active TB, this could result in creation of resistance. This is an important consideration with 4RIF, in view of the very poor treatment outcomes in to Rifampin resistant active TB. However, there was no significant increase in INH resistance with use of INH in numerous trials and cohort studies, nor with RIF in trials and cohort studies. The risk of drug resistance creation from LTBI mono-therapy in children with unrecognized active TB disease is relatively low because of their low bacillary burden. Nevertheless, close surveillance of drug resistance is warranted among children who develop microbiologically confirmed active TB following LTBI therapy in this trial, and is a secondary objective in the adult trial.

1.3.4 Current Canadian[138], and American [107] recommendations for LTBI therapy

Until 1999, 12INH was the standard of care in North America, although 6INH was considered acceptable, because the superior completion rate was considered to offset its lower efficacy [139]. However, based on an analysis by Comstock [140], both the ATS and CTS published revised guidelines in 2000, recommending that 9INH should be the standard of care for LTBI therapy, given its 90% efficacy [140]. 6INH, 2RIF-PZA, and 4RIF were recommended as alternatives. The Paediatric Red Book also recommends 9INH as the preferred or standard regimen, and recommends 6 months RIF as an alternative – for contacts of INH resistant cases.

1.4 RESULTS FROM SYSTEMATIC REVIEWS, AND META-ANALYSES

Several extensive reviews [62] and meta-analyses of trials in HIV infected [141] and uninfected [142] persons have concluded that 6-12 months INH has significantly better efficacy than placebo – in the populations we plan to study. A recent meta-analysis of 5 trials involving a total of 1926 adults randomized to 3INH-RIF or INH concluded that the rate of active TB was similar (4.2% vs. 4.1%) as was the rate of SAE (4.9% vs. 4.8%) [143]. However 83% of subjects received 6INH, which has efficacy of only 40-70% [33,61,65,68,104,128]. Meta-analyses of 2RIF-PZA have been published [118] but this regimen has been abandoned. A recent published meta-analysis of 4RIF concluded that this regimen had significantly lower hepatotoxicity and higher completion rates than 9INH.

1.4.1 Summary of current evidence

Each year, more than 20,000 persons in Canada [54], and at least 300,000 persons in the US [144] initiate LTBI therapy. Over 80% are prescribed 9INH [57], which is considered the standard of care [107,138], but is lengthy, costly, may cause serious adverse events, and has poor completion [70,93,96,98-102]. Of the available recommended alternative regimens, 6INH has efficacy of only 40-70%, and similar risk of adverse events, The 2RIF-PZA regimen was enthusiastically adopted, but then abandoned due to unacceptable toxicity [108,109,112,117,145]. 3INH-RIF appears to have similar efficacy as 6INH, but greater toxicity. This leaves 4RIF, for which there is limited efficacy data, but consistent evidence that safety and compliance are better than with 9INH. With CIHR funding we have completed two trials to compare the 4RIF regimen with 9INH (Section 2.19, and manuscripts in Research Module Appendix). In these and other studies, 4RIF had better compliance

and completion rates [123, 124, 131], lower costs [146, 147], and better safety [123, 146], particularly less hepatotoxicity [148] - the most serious complication with INH.

1.5 HOW WILL THE RESULTS OF THE TRIAL BE USED?

TB is a major global pandemic, and persists in Canada among impoverished and marginalized groups such as urban poor and aboriginal Canadians, as well as immigrants and refugees. Treatment of LTBI has individual and public health benefits, but the current standard of care - 9INH - has serious side effects, and the length of therapy increases costs, yet reduces compliance [93, 97-99, 149], and thereby effectiveness. In children 9 INH has poor completion rates and can cause severe hepatotoxicity, albeit uncommonly. The benefits of a shorter, cheaper, and safer treatment for LTBI would be substantial [130, 150, 151]. In published studies, 4RIF has significantly better completion rates [123, 131], lower costs [123, 146], and better safety [123, 146]. The proposed study will be the largest trial to evaluate mono-RIF therapy for LTBI, and the first trial to evaluate the currently recommended 4 month RIF regimen. However use of 4RIF for children will be limited without information on its acceptability, tolerability and safety - which the proposed trial will provide. The proposed trial will also provide some data on the effectiveness of this regimen in preventing paediatric active TB, albeit with limited power as discussed below. Thus, our proposed study will provide urgently needed data [152, 153] on effectiveness and efficacy of this already recommended (and utilized) alternative LTBI therapy. Involvement of international as well as sites across Canada will provide valuable information on feasibility, tolerability, safety, costs, and effectiveness of 4RIF in different settings, and in adult and paediatric populations - enhancing the potential applicability of results.

1.6 RISKS FOR THE TRIAL PARTICIPANTS

Available evidence, from *millions* of adults and children treated for active TB with RIF in combination with other drugs [154, 155], suggests that RIF is well tolerated and safe. Hepatitis is the most important and potentially fatal complication of INH and RIF-PZA therapy of LTBI. On the other hand, mono-therapy with RIF has been associated with very low rates of hepatitis – in Phase 1&2 [131, 148], and elsewhere [33, 121-124] (RCT Appendix 1 - Table 5). Nevertheless, in view of the 2RIF-PZA experience, where initial trials suggested excellent safety, but unacceptable toxicity was seen with introduction into routine practice, it is prudent to continue to closely monitor the safety of 4RIF in adults and children. This will be done by independent review of all possible Grade 3 or 4 SAE, plus periodic interim analyses of safety during the trial. Patients will be carefully questioned regarding concomitant medications, to identify potential drug interactions, and will be excluded if these can not be managed easily. Mono-therapy of patients with unrecognized active TB, of particular concern in HIV infected patients, may lead to drug resistance, which has serious implications for treatment[152, 153]. As reviewed earlier, this risk appears to be very low, even in children with unrecognized active disease. Nevertheless, the study procedures mandate a thorough medical evaluation to exclude active TB before LTBI therapy is initiated. In all settings we will ensure that study participants have access to all necessary investigations to exclude active TB, including cultures, particularly in HIV infected children. This will include symptom review and chest X-ray. Together these have high sensitivity to exclude active TB in children. To enhance the later applicability of trial results to resource limited settings, we will identify children at risk for active TB using diagnostic algorithms that have been developed and validated in other high TB burden settingsparticularly in hiv infected children In some settings this means budgetary allocations to pay for these investigations if necessary. In the long term, 4RIF will not be useful in resource-limited settings if extensive investigations are needed before starting therapy. Hence, we will develop and evaluate low cost diagnostic algorithms to identify candidates for LTBI therapy likely to have active TB, for use after the trial (RCT Appendix 5). Initial or primary drug resistance can render LTBI therapy ineffective [127]. However rates of RIF resistance rates are very low and much lower than INH resistance in all participating countries (RCT Appendix 1 - Table 6) [156], [157], enhancing the rationale and ethical acceptability of evaluating 4RIF. INH is recommended unless prevalence of primary drug resistance exceeds 50% [107, 158] - clearly not the case in any country, or if the subject is a contact of INH resistant cases INH [107, 138] – an exclusion criteria for this trial. Finally, subjects will be followed two full years after completing 4RIF to ensure early detection of active TB if this therapy fails.

BIOMARKER COMPONENT

The only requirements for this component, in addition to the main randomized trial, will be taking an additional 10 mls of blood pre-treatment, as well as four and nine months after starting treatment, in adults. In total an extra 30ml of blood (equal to 2 tablespoons) will be required over 9 months, and two additional venipunctures, since venipunctures will not be routinely performed at the 4 and 9 month time points. The risks from this amount of blood are nil, although the added venipunctures will cause some discomfort. For adults who were randomized to 4RIF, the blood drawing at nine months will require an extra visit to the clinic, for which they will be compensated.

SECTION 2 THE PROPOSED TRIAL

2.1 STUDY DESIGN

Adult:

We propose a multi-centre randomized 2-arm positive control open-label clinical trial. Patients prescribed standard therapy for LTBI (i.e. 9 months INH) will be approached to participate before they begin therapy. After providing informed consent, subjects will be registered using a web-based system, accessible at all times. This system will verify eligibility and perform immediate on-line randomization, using a

computer generated random sequence, in equal numbers to 4RIF or 9INH - both daily and self administered. Randomization will be stratified by site and in blocks of variable length. The primary end-point will be the occurrence of microbiologically confirmed active TB. Secondary end-points include occurrence of confirmed and probable active TB, Grade 3 or 4 adverse events (SAE), drug resistant active TB, and health system costs. All primary outcomes will be reviewed by an independent 3-member clinical review panel, and all SAE will be reviewed by a different independent 3-member panel. Both panels will be blinded to the study drug, and patient identity.

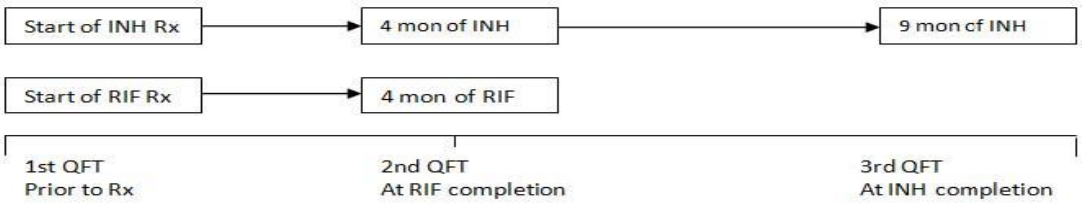
Children:

Eligible children will be up to and including 17 years old, with LTBI, and at increased risk of TB. A child will be defined to have LTBI if they have a positive Tuberculin Skin Test (TST), and active TB has been excluded. A total of 900 children with LTBI who provide assent, and whose parents provide written consent, will be randomized in equal numbers to receive daily self-administered 9INH or 4RIF. Children will be followed by their usual providers during therapy. All adverse events during therapy will be investigated following a standardized protocol. Children will be followed up to 16 months post randomization for the occurrence of confirmed or clinically diagnosed active TB. This study end-point will be judged by a different independent panel of two paediatricians with expertise in the diagnosis and management of paediatric TB, who will review all available clinical, radiographic, and microbiologic evidence while blinded to study drug allocation.

BIOMARKER COMPONENT:

Adults who provide informed consent for the randomized trial will be recruited for this component of the study. Participants will have 10 ml of blood drawn prior to starting on LTBI therapy as well as 4 and 9 months later. This would capture the end of treatment for RIF at the end of month 4, and for INH at the end of month 9 (Figure 1 below). Three of the 10 ml will be used for the QFT-GIT test, the remaining 7 ml will have the serum separated and stored at -80°C for future biomarker studies (Objective 3). All lab assays would be performed in the final year of the study, to enhance cost-effectiveness.

Figure 1: Schematic of study design for Biomarker component



2.1.1 Rationale for an open label trial

The most important departure from the usual methodology of a randomized controlled trial will be the absence of blinding. This is justified by the primary objective to compare the effectiveness of the two regimens which will primarily be determined by the completion rate of therapy. The 9INH regimen has efficacy of approximately 90%[140] if taken fully - making it virtually impossible to demonstrate superior efficacy with 4RIF. However in routine practice fewer than 50% complete INH therapy [70, 93, 96-102], reducing effectiveness to less than 50%. On the other hand, in Phases 1[131] and 2[148], and in observational studies [123, 124] treatment completion rates with 4RIF have been 20-30% higher than with 9INH – similar to trials comparing different durations of the same drugs, in which shorter duration consistently resulted in superior completion [65, 159, 160]. The fundamental rationale for this proposal and the basis for the sample size calculations is that the shorter duration makes improved effectiveness plausible, and detectable. To conduct a fully double blind study, patients assigned 4RIF would take an additional 5 months of similarly coloured and shaped placebo. This would eliminate the most important advantage of 4RIF, and under-estimate its effectiveness. As well, RIF produces reddish discoloration of the urine, so patients assigned to 9INH would take Iron oxide capsules for 9 months. This would render the interventions even less like routine practice, likely reduce compliance even further, and potentially reduce the effectiveness of both regimens to ethically unacceptable levels. Finally, the proposed design is consistent with all other non-placebo controlled trials of LTBI regimens of unequal length – which were all open label, and used microbiologically confirmed active TB as the primary end-point[61, 66, 105, 117, 161 65, 115, 115, 136], as proposed here. The only published double blinded trials of LTBI therapy used placebo regimens of equal length[62-64 104 33, 68, 162]. However, given the consistent benefit of INH in these trials a placebo controlled trial would not be considered ethical.

2.1.2 Rationale for the duration of follow-up

Adult:

The total duration of follow-up will be 28 months. This offers the best trade-off between the greater potential losses and higher costs associated with longer follow-up, and maximizing detection of incident cases of active TB, since disease risk is highest in the first 2-3 years after tuberculin conversion[42, 43], detection of contacts[62], diagnosis of inactive TB [65], or following migration from high to low incidence countries [163 164]. Post-treatment follow-up will be unequal between subjects who complete therapy – 24 months after 4RIF, compared to 19 months after 9INH. This is justified because: (i) We are most concerned about therapeutic failure with 4RIF, given its unknown efficacy.

Hence longer follow-up after therapy will enhance our chances to detect failure in compliant subjects – an outcome of key interest. (ii) On the other hand, given the well documented high efficacy of 9INH, therapeutic failure is unlikely in subjects who complete 9INH, so almost all events of active TB will occur in those who drop-out of INH therapy. Since most drop-outs occur early in therapy [148], length of follow-up after stopping treatment will actually be similar in those actually at risk to develop active TB; (iii) If anything, this approach will favour 9INH slightly, because patients who completed 4RIF will have 5 more months to be re-infected - plausible in high-incidence settings.

Children:

The planned duration of follow-up will be 16 months from the date of randomization, which is shorter than in the adult trial. This is justified by studies from the pre-antibiotic era which reported that active TB developed much more rapidly in children than in adults; in some studies 95% of all cases of active TB following primary infection occurred within 12 months. This will also reduce the risk that children develop TB after re-infection - of concern in high incidence settings. We propose 16 months of follow-up to provide a full year of follow-up after completion of 4RIF, as we wish to detect therapeutic failure in subjects who complete 4RIF, given its unknown efficacy. Post-treatment follow-up will be unequal in subjects who complete allocated therapy. Given the excellent efficacy of 9INH, therapeutic failure is unlikely in subjects who complete 9INH, and more likely in subjects who stop taking 9INH prematurely. Since premature discontinuation of LTBI is usually early in therapy, the length of follow-up after stopping treatment will actually be similar in children who complete 4RIF and children do not complete 9INH. The second motivation for the 16 month follow-up is pragmatic - this will enhance acceptance by study subjects, feasibility for study staff, enhance our retention rate, reduce study costs, and allow the trial to be completed earlier.

2.1.3 Rationale for the primary outcome of safety/tolerability in children:

Although RIF has consistently been safer in adults, and adverse events are typically fewer in children than adults for most TB drugs, an evaluation of safety in children is essential if this regimen is to be adopted in paediatric practice. We will include all Grades of AE because even though Grade 1 or 2 adverse events are not life threatening (for example vomiting), they may be unacceptable to most parents. Hence we believe that milder events, as indicators of tolerability, will be of interest to providers and parents, almost as much as serious (Grade 3 - 4) events.

2.2 INTERVENTIONS

The standard therapy will be daily self-administered INH, 5 mg/kg/day for adults and 10-15 mg/kg/day for children (max=300mg/day) for 9 months (9INH). For adults, dosage will be adjusted if weight is less than 42 kg at 200mg/day. As currently recommended [107, 138, 158] vitamin B6 (pyridoxine) will be given with INH only to patients with risk factors for neuropathy - malnutrition, alcoholism, diabetes, or renal insufficiency or HIV positive. The experimental arm will be daily self-administered RIF, 10 mg/kg/day for adults and 10-20 mg/kg/day for children(max=600mg/day) for 4 months (4RIF). For adults, dosage will be adjusted if weight is 36-49 kg at 450 mg/day or at 300 mg/day for weight of 35 kg and less[107, 138, 165]. For children, dosing for both INH and RIF will be age and weight dependant, with highest doses for infants, and lowest for adolescents. A detailed dose chart - calculating doses by weight and age, and protocols for preparation of medications (crushing pills, mixing suspensions) will be prepared with expert input.

2.2.1 Rationale for the interventions

We have selected 9INH as the standard regimen because: (i) in current guidelines this is the preferred regimen for LTBI[107, 138, 158]; (ii) It is the current standard of practice in North America [97], used in 84% of patients treated for LTBI [57]; and, (iii) INH has consistently had significantly better efficacy than placebo in all trials with study populations similar to the populations we plan to study in adults and children [62, 141, 142]; (iv) in systematic reviews [62], and meta-analyses [141, 142] 9INH has efficacy of as much as 90% [140] if taken properly. We have selected 4RIF as the alternative regimen because this is one of three alternates recommended by the CTS and ATS [107, 138]. Of the two others, 6 months INH had efficacy of 40-69% in several trials [33, 61, 65, 68, 104, 128], and the 2RIF-PZA regimen has unacceptable hepatotoxicity [109, 112, 117, 145]. In our earlier trials who was published and observational studies, 4RIF has had lower toxicity [123, 124, 148], lower cost [147]and better completion [123, 124, 131] than 9INH. There is evidence to suggest the efficacy of 4RIF is as high as that of 9INH, but efficacy has not been adequately assessed. However safety and acceptability have not been studied in children; Rifampin has been used by TB practitioners in all countries of the world for years. Hence it is readily available and familiar to providers; Quality assured RIF produced by generic manufactures now costs only $12 (USD) for a full course of 4RIF, making this an affordable intervention. The experimental regimen will be the same as in the ongoing adult trial, reducing potential errors.

2.3 PROCEDURES OF RECRUITMENT, REGISTRATION, AND RANDOMIZATION

2.3.1 Recruitment:

Adults and children prescribed 9INH therapy and the children's parents will be asked, by their TB care provider, for permission to be contacted by study personnel. Those that agree will be approached to verify eligibility, and obtain signed informed consent for adults, informed assent (children) and parental consent (parents). A section for the biomarker component is included in the main informed consent form for adult only.

2.3.2 Registration

Once subjects have provided informed consent, research staff will access the registration and randomization website via the Internet. This computer based program will be available 24 hours/day and 365 days/year.

2.3.3 Randomization

We propose central, web-based randomization by a computer generated random number producing algorithm, in blocks of varying length (2 – 8 subjects), stratified by centre. Patients who are household contacts of active cases will be allocated to the same regimen as the first member of that household. Because study personnel may recruit more contacts to one arm if they believe it superior, all contacts in one household must be recruited and randomized at once. If additional household members are enrolled at a later date, they will be randomized separately.

2.3.4 Rationale for stratification by centre

The characteristics of the patient populations, their risk of TB, as well as physicians' usual practice – in starting LTBI therapy, or stopping it because of patient intolerance, will vary considerably by centre. Randomization stratified by site should ensure that these and other potential sources of bias by centre are balanced. As well this will ensure that balance is maintained if one or more centres stop enrolling [166], as occurred in Phase 2.

2.3.5 Maintaining Confidentiality

The Web-based registration system is non-nominal. At the time of registration, all patients are assigned a unique study ID number. This will be used to label forms, clinical data, and all case report forms. Only non-nomonal patient information is entered via the Web-based forms. All patient information sent to the data coordinating centre, or reviewed by independent panels, will contain only the study ID. All contact information including names, address, telephone numbers, other contact persons, and full date of birth will be stored at each site, in a secure location, and safeguarded by the site PI. In Canada, lists of participants' names will be forwarded by registered courier to Provincial health authorities to assess if they develop active TB, following procedures approved by provincial privacy commissions. At the international sites the patients' names will be cross-checked against reported TB cases at State, or National level, using appropriate safeguards for patient confidentiality. Protocols will be written for protection of confidentiality; these will be a focus of training and ongoing monitoring of all sites.

2.4 PROTECTION AGAINST BIAS

2.4.1 Preventing bias in ascertainment of the primary outcome

Adult:

Given the absence of blinding, it is important to control for potential bias in ascertaining the primary study outcome. To reduce bias in finding or diagnosing cases, the questionnaire used for follow-up, and the clinical evaluation including diagnostic procedures for active TB will be standardized. The diagnostic process may vary if subjects seek care from non-study providers, but this should not be biased by the LTBI regimen. Each member of the clinical panel will review all available clinical evidence including X-rays, pathology, and microbiology reports, while blinded to subjects' identities, LTBI therapy, and opinions of the other panel members. Each will independently determine if the definition of confirmed active TB is met – ie positive cultures or nucleic acid amplification test for *M. Tuberculosis*, or positive biopsy (see Section 2.8.1 for details). These objective microbiological criteria should not be influenced by patient, nor provider bias, and are the standard outcome for all LTBI trials [33, 61, 65, 68, 104, 128].

Children:

Given the absence of blinding, it is important to limit potential bias in ascertaining the primary study outcome of adverse events. All possible adverse events will be investigated using standardized protocols. A 3-member independent panel will review each event, blinded to the study drug, and opinions of the other panel members to judge relationship to study drug, and grade severity. Grading will be standardized - using the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html), except hepato-toxicity will be based on ATS guidelines. **2.4.2 Preventing bias in ascertaining secondary outcomes**

Adult:

The secondary outcome of probable TB, defined as compatible clinical and radiographic features will be judged by the same 3-member clinical review panel, using the same approach as above. All possible Grade 3 or 4 SAE will be investigated using standardized protocols, and graded according to ATS guidelines for hepato-toxicity [155], or the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html) for all others. Grading, and attribution of cause, will be performed by three independent reviewers blinded to the identity of patients, study drug, and opinions of the other reviewers. We will ascertain costs based on visits, tests and other health services – all of which are quite objective. To further minimize bias, the laboratory technicians who perform cultures and drug sensitivity testing, and the cost-effectiveness analyst will be blinded to study regimen.

Children:

Completion will be based on dosage count (pill count or amount of liquid suspension). During treatment phase, the pills or suspension

dispensed at each interval will be standardized. The personnel counting dosages will be unblinded, so will be instructed to simply count and record doses remaining in the bottles, without interpretation, or enquiry to when, or how many were dispensed, nor to alter the count recorded in light of patients' remarks. If patients forget to bring the medication bottle for a visit, a new supply of medication will be dispensed in a new bottle, and they will be asked to bring both bottles the next visit (and so on, for subsequent visits if they keep forgetting). If a bottle is never returned, then it will be assumed those doses were never taken. This will likely result in an under-estimate of doses taken, but will be more conservative.

To reduce potential bias in detecting active TB cases, the questionnaire used for follow-up, and the diagnostic procedures for active TB will be standardized. As well, the laboratory technicians who perform cultures and drug sensitivity testing will be blinded. The diagnostic process may vary if subjects seek care from non-study providers, but this should not be biased by the LTBI regimen. All final diagnoses of active TB will be based on the judgement of the independent clinical review panel of two paediatricians with internationally recognized expertise in paediatric TB. They will review all available clinical evidence including X-rays, pathology, and microbiology reports, while blinded to subjects' identities, and LTBI therapy.

2.5 INCLUSION AND EXCLUSION CRITERIA

2.5.1 Inclusion criteria Adult:

Adults (age ≥ 18) with documented positive TST as defined below and prescribed 9INH for LTBI, following authoritative recommendations [107, 138]:

Note: In the absence of a TST test, a positive QFT (or T-Spot) (according to manufacturers recommendations) (see screening, recruitment and randomisation procedures) is equivalent to a TST of 10 mm.

1. HIV positive , OR to start TNFa inhibitors, OR on transplant anti-rejection medications.(TST ≥5 mm or QFT +)
9. Close contact: ≥4 hours contact per week, for ≥ 1 week with person with active pulmonary TB. (TST ≥5 mm or QFT +)
10. Apical/upper lobe fibronodular disease with area >2cm^2 (shown in RCT Quick Guide) (TST ≥5 mm or QFT +)
11. Documented tuberculin conversion within two years. (Increase ≥ 6 mm, with subsequent TST ≥10 mm or QFT +)
12. Diabetes, renal failure, or immuno-compromised from medical condition or therapy (TST ≥10 mm or QFT +)
13. Casual contact: contact of <4 hours/week, with a person with smear positive pulmonary TB. (TST ≥10 mm or QFT +)
14. Tuberculin conversion within 2-5 years. (Increase of 10 mm or more with subsequent TST ≥10 mm or QFT +)
15. Have (1) **TWO** of the following **four** factors if TST = 10-14mm or QFT +,

OR (2) **ONE** factor if TST ≥15mm:

a. Arrival in Canada, Australia, or Saudi Arabia in the past 2 years from countries with WHO estimated incidence greater than 100 per 100,000 (these are listed in the RCT Procedure guide - Appendix 5)
b. BMI <19 (BMI calculations, see RCT Procedure guide);
c. Any abnormality on chest x-ray compatible with past-TB infection e.g. calcified granuloma, or hilar lymph nodes, costo-phrenic angle blunting - other than fibronodular disease above.
d. Cigarette smoking (at least a half pack per day) currently.

In the low and middle-income countries, LTBI therapy will usually be offered only to patients in categories 1& 2[158], because of resource limitations, resulting in substantial differences in risk of active TB among untreated patients at the different sites. Balanced allocation of risk groups to the two study arms will be maintained by site-stratified randomization.

Children:

Children (age <18) with documented positive TST as defined below and prescribed 9INH for LTBI, for the indications below, as currently recommended:

Note: In the absence of a TST test, a positive QFT (or T-Spot) (according to manufacturers recommendations) (see screening, recruitment and randomisation procedures) is equivalent to a TST of 10 mm.

1. HIV positive (TST ≥5 mm or QFT +).
2. Age 5 or less (TST ≥5 mm or QFT +).
3. Other reason for immuno-compromised state - such as therapy for malignancy or post-transplant (TST ≥5 mm or QFT +).
4. Contact: with adult or adolescent with active contagious pulmonary TB. (TST ≥5 mm or QFT +)
5. Have **both** of the following factors if TST = 10-14mm or QFT + or **one** factor if TST ≥15mm :
    a. Arrival in Canada, Australia, or Saudi Arabia in the past 2 years from countries with estimated annual incidence of active TB greater than 100 per 100,000
    b. Body mass index (BMI) less than 10th percentile for their age.

Interferon gamma release assays (IGRA's) are ex-vivo tests of immune response to TB antigens, that have been adopted in some centres as alternatives to the TST, although WHO has recently recommended IGRAs should not be used to replace the TST in low and middle-income countries. If an eligible child undergoes a commercially available IGRA (the Quantiferon-Gold or T-Spot.TB), instead of a TST, and the result is positive, then they will be considered eligible. If both TST and IGRA are done, then the TST result will be used to determine eligibility.

The TST may be negative for up to 8 weeks after primary infection, before adequate cell mediated immunity develops. Because of this, current practice is to begin LTBI treatment therapy immediately for children ≤ 5 years old, even if TST negative. After 8-10 weeks the TST is repeated; LTBI therapy is continued if now TST positive, and stopped if still negative. Providers may continue therapy in very young, HIV infected or malnourished children. We propose to enrol TST negative children aged ≤ 5, if the treating physician prescribes LTBI therapy, because: 1) primary endpoints are still relevant, and measurable in this group; 2) acceptability and completion in this sub-group are of particular interest; 3) children that have new primary TB are at particularly high risk to develop disease (this is the rationale for their treatment). If the treating MD stops therapy because the TST is negative after 8-10 weeks, these children will be excluded from the analysis of treatment completion, but included in the incidence density analysis (person-time) of tolerability and safety.

Biomarker component:

1. Adults (age ≥ 18) who will be recruited in RCT phase 3 and is willing to take part.

2.5.2 Exclusion criteria

Adult:1. Patients who were contacts of TB cases known to be resistant to INH, RIF, or both (i.e. MDR)[107, 138, 127].

2. Known HIV-infected individuals on anti-retroviral agents whose efficacy would be substantially reduced by Rifampin, unless therapy can safely be changed to agents not affected by Rifampin (listed in RCT quick guide - Appendix 5).
3. Pregnant women - Rifampin and INH are considered safe in pregnancy [138, 167], but therapy is usually deferred until 2-3 months post-partum to avoid fetal risk and the potential for increased hepato-toxicity immediately post partum [168].
4. Patient on any medication with clinically important drug interactions with INH or RIF, which their physician believes would make either arm contra-indicated. An updated list of clinically important drug interactions is in the RCT Quick Guide (Appendix 5). This includes women taking hormonal contraceptives who will not take alternative contraception.
5. History of allergy/hypersensitivity to Isoniazid or to Rifampin, Rifabutin or Rifapentine.
6. Active TB. Patients initially suspected to have active TB can be randomized once this has been excluded.
7. Persons who have already started LTBI therapy.

Children:

Criteria 1 to 6 are the same as for adults.

Criteria 7- Prior complete LTBI therapy or if children have taken >1 week and are still taking the treatment.. Children will be eligible if they took an incomplete LTBI therapy (less than 80% of recommended total dose) but > 6 months ago.

2.5.3 Rationale for exclusion criteria

Exclusion criteria 1-6 enhance the safety of participants, while criterion 7 is to avoid unnecessary therapy. We are interested in the real world application of Rifampin, and so will include persons potentially at risk for non-completion or for adverse events. To obtain a realistic estimate of safety and tolerability, no patient will be excluded on the basis of age nor history of TB therapy, liver disease, alcohol use, or other medication use (except as specified above). If the treating physician prescribed LTBI therapy, they must have concluded the benefits of therapy outweighed the risks. Similarly, to obtain a realistic estimate of effectiveness, patients at risk of non-completion (homeless, alcoholic, and drug use) will be included. An additional reason for these inclusion criteria is to enhance comparability with earlier trials [169] - which involved subjects who were older[33, 65, 161], HIV infected[68, 104], or had other co-morbidities[62].

2.6 DURATION OF TREATMENT: (see section 2.2 – Interventions)

2.7 FOLLOW-UP AND DATA GATHERING

2.7.1 Initial evaluation

Adult:

The initial evaluation, completed by study personnel, will include demographic information, past medical history, reasons for LTBI therapy, tuberculin reactions, chest X-ray findings, other investigations, and HIV status if known. In contrast to patients with active TB, HIV testing of LTBI candidates is not considered essential for patient care [107, 138, 158]. Hence HIV testing will be offered to all subjects, with appropriate counselling, although it will not be a required test for inclusion in the study. The need for other investigations is at the discretion of the provider, except for subjects with chest X-ray abnormalities with lesions ≥ 2cm^2, or close contacts with symptoms or X-ray abnormalities, in whom sputum specimens must be sent for AFB smear and culture [107, 138]. If investigations to exclude active TB are performed, patients can not be enrolled until results are available. We have developed a draft algorithm to identify and investigate subjects with possible active TB (RCT Appendix 3). This will be evaluated and validated to facilitate the use of LTBI therapy in resource-limited

settings after the trial is completed.

Children:

Prior to randomization and initiation of LTBI therapy, a thorough medical evaluation to exclude active TB will be completed, with a particularly careful examination in HIV infected children. This will include symptom review and chest X-ray, which together have high sensitivity to detect active TB in children. In the presence of chest X-ray abnormalities or symptoms, sputum specimens must be sent for AFB smear and culture, and children can not be enrolled until results are available. To enhance the applicability of trial results to resource limited settings, we will use diagnostic algorithms that have been developed and validated in other high TB burden settings. In contrast to patients with active TB, routine HIV testing of LTBI candidates is not recommended, but will be offered to children with any risk factors for HIV infection such as another member of the household has HIV infection.

2.7.2 Follow-up during treatment

We wish to ascertain treatment effectiveness under routine programme conditions. Therefore, follow up will be in line with standard practice, and conducted by the initial treating physicians and TB clinic staff, meaning that visits will be monthly for the first 2 months, then every 2 months (minimum) thereafter for both treatment regimens. Blood count (CBC), and liver transaminases will be checked pre-treatment for adults and children and at the first follow-up visit for adults only. Blood count for children not done routinely during treatment phase follow-up, unless symptoms or problems arise [213]. Patients will be encouraged to call, or see their TB therapy provider or TB clinic staff, if they develop any new symptoms. Concomitant treatment is a potential problem with un-blinded therapy [166] but given the specificity of anti-TB drugs it is highly unlikely that any such therapy would be given, unless active TB was diagnosed. To monitor drug interaction, for medications whose drug levels can be monitored, these will be measured at 0, 2, 4 and 8 weeks, along with any related dosage changes. For anti-coagulants, anti-diabetics, anti-hypertensives or lipid lowering agents the clinical end-points will be monitored - at 0, 2, 4 and 8 weeks of therapy, plus related dosage changes.

2.7.3 Post treatment follow-up

After treatment is finished (or discontinued) post-treatment follow-up will begin. Follow-up will be every 3 months until 28 months for adults and until 16 months for children post randomization on the date corresponding to 3 monthly intervals after randomization. Follow-up frequency is because of experience gained with Phase 2 participants – frequent contacts are needed, even if brief, to enhance retention, because so many move, change telephones, or lose interest. Our objective is to achieve a drop-out rate less than 10%.

The rational for 16 months follow up for children was given in section 2.1.2

2.7.4 Maintaining high quality data:

2.7.4.1 Training:

Adult:

At the start, we will hold a two day training course for all investigators and research coordinators (see Appendix 7- Time-Table) to review eligibility criteria, ethics, consent procedures, protection of confidentiality, registration, randomization, reporting of serious adverse events, and follow-up procedures during and after treatment. Particular attention will be paid to procedures to minimize drop-outs, investigation and management of SAE during therapy, and ascertainment of active TB. Staff at new sites will receive additional intensive training at the study coordinating centre

Children:

At the start of this trial, we will hold a two day training course at the coordinating centre for all investigators and research coordinators, to review eligibility criteria, ethics, consent procedures, protection of confidentiality, registration, randomization, reporting of serious adverse events, and follow-up procedures during and after treatment. Emphasis will be placed on the differences of the paediatric trial design, and the diagnosis of active TB in children. On-site training (1-2 days at smaller sites, and up to 1 week at larger) will be provided by the study coordinator (Karen Hornby) when each site initiates paediatric enrolment. This training will be shorter than for the adult trial, given the overlap in staffing expected, and similarities in design and methods to the adult trial.

2.7.4.2 Supervision: The PI will visit all new sites within three months after beginning randomization, and all sites annually. The study coordinator will visit new sites at the time of trial initiation, all sites every three months in year 1 and every six months thereafter. We will verify that patients are approached, informed, and provide consent correctly. We will ascertain that documentation of IRB correspondence and consents are complete, confidentiality is protected, and that data entered in databases, or using the Web-based programmes, corresponds to source documents. Verbal feedback will be provided to staff immediately, and written reports will be reviewed with the site investigators and staff, and the principal investigator. To enhance the cost-effectiveness of this proposal, supervision and monitoring for adult and paediatric trials at each site will be completed at the same time.

2.7.4.3 External Audit: We wish to follow all guidelines for Good Clinical Practice (GCP) for randomized trials. For all Canadian sites, we plan two independent audits – the first 6-9 months after starting enrolment, and the second 18 months later. Both will be performed by a professional clinical research associate experienced in auditing pharmaceutical sponsored trials. For budgetary reasons, the professional

auditor will make only one visit to each international site - one year after beginning randomization. The PI will make the second audit visit 12 months later. These audits will review all SOPs - in order to ensure they conform to GCP, and will be used to enhance the monitoring skills of the study coordinators (K Hornby and C Valiquette) who will subsequently perform similar audits of all sites during their visits.

2.7.4.4 Electronic databases - web based and local: The web based initial registration incorporates all information from the initial case report form. All SAE and active TB will also be reported using non-nominal web-based forms. To collect all other treatment phase, and post-treatment follow-up information, site staff will use local databases. The data will then be transferred –in non-nominal form- to the coordinating centre. All web-based forms and randomization software, as well as local databases were developed in Phase 2, except for reporting the primary outcome of active TB - which will be added. All web-based forms and randomization software as well as databases developed for the adult trial will require relatively minor modifications for the paediatric trial.

2.8 THE PRIMARY AND SECONDARY OUTCOMES IN ADULTS:

2.8.1 Definition of the primary outcome

Confirmed active TB during 28 months after randomization will be defined as a positive culture for *M. tuberculosis*, positive Nucleic acid amplification test for M TB complex, or caseating granulomas in a biopsy from any site. Positive AFB smears will be considered false positive if cultures are negative, but will be considered confirmatory, if cultures failed (for example if contamination or other technical problem occurs).

2.8.2 Definition of the secondary outcome of probable active TB

Probable active TB during 28 months after randomization will be defined as a compatible abnormal chest X-ray plus clinical symptoms, which improve following treatment for active TB, as judged on blinded review by a majority of the independent clinical review panel members.

2.8.3 Other secondary outcomes

See below for compliance, serious adverse events, costs, and drug resistance.

2.8.4 THE PRIMARY AND SECONDARY OUTCOMES – DEFINITIONS FOR CHILDREN:

2.8.4.1 Intolerability/safety - adverse events (AE):

The outcome of intolerability/adverse events (or the 'inverse' of safety) will include adverse events of all levels of severity (Grades 1 to 4) that resulted in permanent discontinuation of study drug, that were judged probably related to the study drug by a majority (2 out of 3) of independent review panel members.

2.8.4.2 Treatment completion:

Treatment completion will be defined as consumption of 80% or more of the recommended total doses. This threshold was selected because of evidence of high levels of protective efficacy among subjects who took at least 80% of doses of INH in a large scale LTBI trial. We will allow 33% more time to complete therapy meaning a maximum of 23 weeks for RIF and 52 weeks for 9INH, as in other studies.

2.8.4.3 Active TB:

Active TB during 16 months after randomization will include confirmed and probable active TB. Confirmed active TB will be defined as a positive culture for M. tuberculosis, positive Nucleic acid amplification test for M TB complex, or caseating granulomas in a biopsy from any site. Probable will be defined as a compatible abnormal chest X-ray plus clinical symptoms, which improve following treatment for active TB, as judged on blinded review by the independent clinical review panel members.

2.8.4 Drug resistance: Resistance will be defined based on the critical thresholds used by the lab performing the DST.

2.9 (A) MEASURING THE PRIMARY AND SECONDARY OUTCOMES IN ADULT

2.9.1 (A) The primary outcome of active TB

Our primary method to detect active TB will be active follow-up. Participants will be instructed to contact study personnel if symptoms suggestive of active TB arise – during, or after completion of therapy. During LTBI therapy, subjects will be questioned at each follow-up visit for symptoms suggestive of active TB. After therapy has been completed they will be contacted every three months by study personnel until 28 months after randomization. This will be done primarily by telephone in Canadian and Australian sites, by direct patient visits in Brazil and Africa, and both methods in Korea and Saudi Arabia. At each contact, standard questions (listed in RCT Procedures – Appendix 5) will be asked about current symptoms, and if they were diagnosed with TB since last contacted. Any patients with symptoms suggestive of active TB will be evaluated promptly by study personnel following a standardized protocol, including X-rays, sputum AFB smears and cultures. If subjects are diagnosed with active TB elsewhere, information will be collected regarding date of diagnosis, date and type of treatment, treating physician, and health facility. Permission will be sought to obtain clinical, laboratory, and treatment information, and copies

of relevant X-rays from the treating physician.

2.9.1.2 (a) Verifying the diagnosis of active TB: An independent 3-member clinical panel will review X-rays, and all clinical and lab information. All members are Montreal-based chest specialists with more than 20 years experience, including many patients with active and latent TB (see Research Module Appendix 1); none are co-investigators. Panel members will independently diagnose each case as confirmed, probable, or unlikely TB, as defined above, blinded to patient identity, LTBI regimen, and opinion of treating physician or other panel members. Differences will be resolved by consensus.

2.9.1.3 (a) Assessing completeness of ascertainment of active TB: To verify the completeness of ascertainment of active TB with our proposed method of follow-up, we will send a list of all randomized subjects to public health officials responsible for the TB registry in each jurisdiction. They will match subjects names with nominal registries held at provincial, state, or national levels. This is included in the consents for Phases 2, and 3 (Research Module Appendix 1). This will allow us to ascertain any under-estimate of the primary outcome, although patients with clinically diagnosed TB, and those who developed TB after leaving the province/state where enrolled may be missed. We will use the same verification procedure for the international sites. In Canada, provincial registries are almost 100% complete for microbiologically confirmed cases, but the completeness, accuracy and comparability of the TB registries at the international sites are unknown. However any possible differences in detection between sites should not bias the comparison of regimens, given the stratified randomization by site.

For the Canadian sites we will ascertain outcomes of consenting participants who were lost to follow-up, through the provincial health administrative databases. We will forward the names of these subjects, to the provincial health authorities 28 months after randomization, to verify whether they have died (if so, date of death), or moved out of province (if so, when). This will enable us to ascertain outcomes of such subjects, and to examine potential bias due to these drop-outs. **2.9.2 (A) The secondary outcome of compliance during treatment**

Compliance with treatment is an important modifier of treatment effect, and must be measured to perform the planned efficacy analysis among the sub-groups who take therapy per protocol. In Phases 1 & 2 we used the Medication Event Monitoring System (MEMS), to record pill taking behaviour. However we plan to use pill counts as our primary method of assessing compliance because: (i) pill counting is inexpensive, and feasible for all programmes – hence results can be more easily reproduced elsewhere; (ii) in Phase 2 pill counts were highly concordant with the MEMS records (iii) the MEMS is very expensive, and so is unlikely to be adopted in practice – even in North America; (iv) in a previous trial, pill counting had excellent predictive value for risk reduction [65].

2.9.3 (A) The secondary outcome of serious adverse events (SAE)

At each follow up visit patients will be questioned and examined for evidence of adverse events. Prior to beginning therapy and at the first follow-up visit, CBC, and liver transaminases (AST and ALT) will be tested. As in Phase 2, (see Section 2.19) site investigators will file an initial web-based SAE report if therapy is discontinued because of patients' symptoms or lab abnormalities. They will be investigated and managed following standardized protocols developed in Phase 2 (RCT Appendix 2), based on our published experience [170], recent reviews [154, 155] and authoritative guidelines [138, 155]. Adverse events will be graded as suggested in guidelines by the American Thoracic Society for hepato-toxicity [155], and the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html;). As in Phase 2 we will have an independent 3 member panel (Dr Rick O'Brien, Dr Mike Lauzardo, and Dr Wendy Cronin - letters in Research Module Appendix 1). This panel will review all possible SAE – defined as events that lead to permanent physician discontinuation of study drug, without knowledge of study drug, nor opinions of neither other panel members nor treating physician. They will judge type, severity, and probability of cause of the SAE. Differences will be resolved by consensus. Results of this blinded review by the independent panel will be considered the final diagnosis for type, severity, and relationship to the study drugs. Detection of Grade 3-4 reactions may be greater than in routine practice, but this should occur equally for both arms, and enhance research subjects' protection. Unreported adverse events cannot be reviewed - leaving room for provider bias. This may occur with minor intolerance, (which will be termed patient non-compliance). However, if the provider stops therapy because of adverse events, this must be reported by investigators, and investigated as above. The anticipated number of participants in each arm will provide substantial power to detect relatively small differences in occurrence of SAE. This should provide the opportunity to detect less common SAE, such as hematologic complications, or ascertain if differences occur within subgroups (e.g. HIV infected). (See also Section 2.18).

2.9.4 (A) The secondary outcome of drug resistance among cases of active TB

All positive mycobacterial cultures from subjects who develop active TB within 28 months post randomization will be sent to reference TB laboratories for identification and drug susceptibility testing. All international sites have access to reference TB laboratories, which participate in external quality control programmes with WHO supra-national reference laboratories.

2.9 (B) MEASURING THE PRIMARY AND SECONDARY OUTCOMES IN CHILDREN

2.9.1 (B) Adverse events (AE): At each follow up visit children will be questioned and examined for evidence of adverse events. Suspected AE will be investigated and managed following standardized protocols, developed for the adult trial based on our published experience, recent reviews and authoritative guidelines. These have been revised for children with input from expert paediatricians (Dr Marais). We will have an independent 3 member panel (Dr's O'Brien, Lauzardo, and Cronin) review all possible AE – defined as symptoms,

signs, or lab abnormalities that lead to physician discontinuation of study drug. Panel members will judge type, severity, and probability of cause of the AE, without knowledge of study drug, nor opinions of other panel members. Adverse events will be graded as suggested in guidelines by the National Cancer Institute Common Terminology Criteria for Adverse Events v2.0 (at http://ctep.info.nih.gov/reporting/ctc.html;), or the American Thoracic Society for hepato-toxicity. The opinion of 2 out 3 independent panel members will be considered the final diagnosis for type, severity, and relationship to the study drugs. Detection of adverse reactions may be greater than in routine practice, but this should occur equally for both arms, and enhance research subjects' protection. Unreported adverse events cannot be reviewed - leaving room for provider bias. However, if the provider stops therapy because of a suspected adverse event, site personnel must complete a web-based AE report, and investigate this as above.

2.9.2 (B) Completion of treatment: We plan to use dosage counts as our primary method of assessing compliance because: 1) this is feasible for all programmes – hence results can be more easily reproduced elsewhere; 2) in our earlier trials, dosage counts were highly concordant with records of the Medication Event Monitoring System (MEMS); this system closely records pill taking behaviour, but is very expensive, and unlikely to be adopted in practice – even in North America; 3) in a previous trial, results from dosage counts were strongly associated with estimates of risk reduction.

2.9.3 (B) Active TB: Our primary method to detect active TB will be active follow-up. Parents and children will be instructed to contact study personnel if symptoms suggestive of active TB arise – during, or after completion of therapy. During LTBI therapy, and every three months after LTBI therapy up to 16 months post randomization, subjects will be questioned regarding symptoms suggestive of active TB. Standard questions (listed in RCT Procedures – Appendix 5) will be asked about current symptoms, and intercurrent new medical problems (such as TB). Any patients with symptoms suggestive of active TB will be evaluated promptly by study personnel using a standardized protocol, including X-rays, sputum AFB smears and cultures. If subjects are diagnosed with active TB elsewhere, information will be collected regarding date of diagnosis, date and type of treatment, treating physician, and health facility. Permission will be sought to obtain clinical, laboratory, and treatment information, and copies of relevant X-rays from the treating physician.

2.9.1.2 (b) Making an unbiased diagnosis of active TB: An independent 2-member clinical panel (Drs Marais and Schaaf of Stellenbosch Univ, Capetown) will review all clinical, radiologic, and lab information. Both are paediatricians with internationally recognized expertise in diagnosis and management of paediatric TB; neither are investigators at enrolling sites. Panel members will independently judge each case as confirmed, probable, or unlikely TB, as defined above, while blinded to patient identity, and LTBI regimen. Differences will be resolved by consensus.

2.9.1.3 (b) Assessing completeness of ascertainment of active TB: In addition, at the end of the study , we will check cross-check names of study subjects from each site with the corresponding nominal national or provincial/state TB registries, to ascertain if any study subjects were diagnosed with active TB, and if so information about them will be collected and reviewed as above.

2.10 (B) Surveillance of drug resistance among cases of active TB: All positive mycobacterial cultures from subjects who develop active TB within 16 months post randomization will be sent to reference TB laboratories for identification and drug susceptibility testing. All international sites have access to reference TB laboratories, which participate in external quality control programmes with WHO supra-national reference laboratories.

2.10 MEASURING HEALTH SYSTEM COSTS

We will gather information on all health services (visits, consults, tests, drugs) used by participants' during treatment, and if they develop active TB, as an integral part of the local and web-based data-bases. Hence this information will be routinely gathered as part of the trial, as was done in Phase 2. Investigators at each site will provide site specific estimates for values (costs) for all these health services, using methods that we have used in earlier studies [54,171-173]. (Details in RCT Appendix 4).

2.11 SAMPLE SIZE REQUIREMENTS FOR ADULT:

Our primary objective is to test whether 4RIF has superior effectiveness compared to 9INH in reducing occurrence of confirmed active TB, based on a planned intention to treat analysis. The number required depends upon the estimated rate of active TB in treated, and untreated patients, and the expected completion rates with 9INH. The expected rate of TB is hard to predict, since we will enrol patients at a wide range of increased risk. In the international sites, where most patients will be contacts (risk over 2 years of 3% [62,174] up to 8-10% [63,175]), or HIV infected (**annual** risk 3-8% [20,21]), cumulative incidence if untreated (or non-compliant) should exceed 5%, but may be only 2% in untreated subjects in Canada. Hence we assume an average cumulative incidence of 3% among untreated. Based on the assumption that 9INH has 90% efficacy if completed [140], but only 50% completion rate [70,93,96-102], and 4IRF efficacy is 90% the required sample size, calculated using Poisson distribution, would be 2,898 participants with full follow-up in each group (see Table S1 below). If re-calculated using the binomial distribution (and *http://stat.ubc.ca/~rollin/stats/ssize/b2.html*) the sample size required would be 2% to 5% larger. Smaller differences in completion will reduce power, but higher event rates (that are certainly plausible) will substantially increase power.

Table S1: Sample size required to detect superior effectiveness of 4RIF compared to 9INH

(calculated using Poisson distribution [176], assuming alpha=0.05 and two-sided tests)

Expected cumulative incidence of TB over 28 months after randomization			N (per group) to detect difference, with power of:		
No therapy	**9INH** (Completion 55%, Effectiveness 49.5%)	**4RIF** (Completion 80%, Effectiveness 72%)	**60%**	**70%**	**80%**
3%	1.49%	0.84%	2,648	3,337	4,243
4%	1.98%	1.12%	2,013	2,537	3,226
5%	2.48%	1.4%	1,598	2,013	2,560
No therapy	**9INH** (Completion 50%, Effectiveness 45%)	**4RIF** (Completion 80%, Effectiveness 72%)	**60%**	**70%**	**80%**
3%	1.65%	0.84%	1,809	2,279	2,898
4%	2.20%	1.12%	1,357	1,709	2,173
5%	2.75%	1.40%	1,086	1,368	1,739

Members of the same household will be randomized to the same regimen. When cluster randomization is used, sample size must be adjusted accordingly because members of the same cluster are no longer independent observations [177]. Usually, the required sample size is inflated by a factor of $1+(m-1)*ICC$; where m is the average cluster size and ICC is the intra-cluster correlation coefficient which denotes how similar subjects from the same cluster are with respect to their risk of developing the outcome, compared to those in other clusters [178]. Given that the cluster sizes will vary (in Phase 2, 20% of subjects were household contacts, of whom 60% were single contacts, and 40%, or 8% of all participants, were in groups of 2-4 persons - with an average number of 2.5 contacts), we adjusted for this via the method described in [178]. This calculates the design effect as: $1+[(cv^2+1)m-1]*ICC$ where cv is a ratio of the standard deviation of the cluster sizes, m and ICC as before. Even assuming an ICC as large as 0.1 (ICC is typically 0.05-0.12 for spouse pairs [179]), this only results in a design effect of 1.03 - meaning a 3% increase to 2985 per group. Allowing for 10% loss to follow-up the number must be increased to 3283 per arm. Patients will continue to be followed even if they stop therapy themselves (i.e. non-compliance), or their physician stops therapy (e.g. pregnancy or adverse events). Because 847 subjects, enrolled in Phase 2 of this trial, are still being followed for occurrence of active TB using the same methods as proposed here, the total of number of new participants required in this phase can be reduced to 5720. However, given the greater than expected loss to follow-up in Phase 2, we will increase this to a total of 6,000 enrolled in Phase 3.

We have assumed 4RIF efficacy of 90%, based on available evidence. As shown below, if 50% of the 2,898 randomized to each group complete therapy and 28 months follow-up, this would provide more than 90% power, to confirm **non-inferior** efficacy of 4RIF, if the **non-inferiority** margin was 25% - equivalent to a minimum efficacy of 4RIF of 65%. (In other words, we would declare 4RIF **non-inferior** to 9INH if the efficacy of 4RIF was not more than 25% worse than 9INH.) This efficacy has been considered sufficient for authoritative recommendations of 6INH [107, 138], which has had efficacy of 40-69% in trials [33, 61, 65, 68, 104, 128].

Table S2: Sample size to assess non-inferiority of 4RIF efficacy

(calculated using alpha=0.05 and one-sided test using methods suggested by Blackwelder [180])

2.11.1 SAMPLE SIZE

Expected cumulative incidence of TB		Tolerated difference		Number (per group) required to provide power of	
Untreated	**9INH**	**Δ (25%)**	**Maximum Event rate with 4RIF**	**80%**	**90%**
3%	0.3%	0.75%	1.05%	658	911
4%	0.4%	1%	1.4%	493	683
5%	0.5%	1.25%	1.75%	394	546

REQUIREMENTS FOR CHILDREN: (FOR DETAILS REFER TO APPENDIX 11)

To conclude that the rate of Grade 1-4 adverse events was not significantly worse with 4RIF (ie to conclude non-inferiority of 4RIF for AE), with 80% power, and a maximum tolerated difference of 5%, we would require 279 subjects in each group if the total rate of these

events was 6% with 9INH.

To detect that 4RIF has a 10% better completion rate with 80% power, and alpha=0.05, would require 356 subjects per group, if 60% of children complete 9INH. Based on Tables 2 and 3, **our target sample size will be 356**-as this will provide adequate power for our primary objective and to adequately assess completion. All children in the same household will be randomized to the same regimen. Because members of the same cluster are no longer independent observations, the required sample size will be inflated by a factor of $1+(m-1)*ICC$; where m is the average cluster size and ICC is the intra-cluster correlation coefficient. Given that the cluster sizes will vary and using the number and size of clusters of household contacts randomized in our earlier trial, we adjusted using the method described in. This calculates the design effect as**:** $1+[(cv^2+1)m-1]*ICC$ where cv is a ratio of the standard deviation of the cluster sizes, m and ICC as before. Even assuming an ICC as large as 0.3 (ICC is typically 0.05-0.12 for spouse pairs, this results in a design effect of 1.10 - meaning a 10% increase to 392 per group. This will be increased by 5% to **411 per group** to account for subjects that never start therapy, who will not be at risk for adverse events.

2.11.2 SAMPLE SIZE CONSIDERATIONS FOR BIOMARKER COMPONENT

A sample size of 750 adults would provide adequate power to address Objective 1, and, this will also provide ample power for multi-variate modeling to address Objective 2. Since the required sample size for Objective 1 is larger, we plan to enrol 750 analyzable subjects. We will increase this number by 5% to account for potential missing values (test failure, inability to draw blood, and drop-outs) to 788 total participants. As many as 5% of patients may be QFT-GIT negative at enrolment (since eligibility in the parent trial is defined based on a positive TST, and there is always some discordance between QFT-GIT and TST . To account for this possibility, we will increase this by another 5% to **a final target sample size of 826** in order to identify 788 initially QFT-GIT positive. These sample size computations are based on changes in IFN-γ responses, but we would expect the same to apply to changes in IP-10 responses, based on published literature.

2.12 PLANNED RECRUITMENT RATE: (see also Section 2.15)

Adult:

At the Canadian sites, we anticipate 455 subjects enrolled annually, based on an expectation of similar enrolment at continuing sites, and the addition of Vancouver. The Vancouver site is the largest TB clinic in Canada with more than 800 persons treated for LTBI annually. Given the allocation of the same budget as for Montreal and with 33% more patients, it seems realistic to expect similar recruitment rates as in Montreal. We plan to double the capacity in Brazil by doubling staff and doubling the number of clinics at which patients will be enrolled. We anticipate little change in enrolment in Saudi Arabia. Two new sites will be added in West Africa; both have substantial experience and infrastructure for the conduct of randomized trials of active TB. We anticipate a rapid start-up with an average of 500 enrolments annually from both sites (letter from Dr. Lienhardt - Appendix 1). In Seoul, Korea four University hospitals and the very busy clinic at the Korean Institute of TB (KIT) will be added, under the direction of Dr Woojin Lew (Director of KIT - see letter in Appendix 1). Investigators at KIT were recently awarded a major grant for a 10 year longitudinal study of 3,000 close contacts. It is anticipated that at least 500 contacts with LTBI will be recruited annually at this site. In Australia and Saudi Arabia, the patient volumes are much lower, but addition of these two sites will be valuable because of the high quality TB programmes, and potentially greater generalizability of results to a broader range of settings. In addition both sites will cover all local costs – hence only travel costs for meetings, training, and supervision will be needed (see letters from Dr Marks, and Drs Al Jahdali and Memish - Appendix 1). Dr. Menzies will spend three months during years 1 and 2 in getting the study initiated at all these sites. (This plan is supported by his department Chair - see Dr Eidelman letter in Appendix 1). If all sites enrol a total of 1,950 participants annually (see section 2.15), we should complete enrolment within 3 years, although we have planned recruitment over 3.5 years. And, as in Phase 2, enrolment will be monitored closely, and corrective actions taken promptly - including addition of sub-sites, and, if necessary, shifting operating budgets from under-performing sites.

Children:

At the five sites in Canada, Australia and Saudi Arabia, we anticipate a total of 100 subjects enrolled annually. The patient populations at these sites are predominantly foreign-born children; this should enhance generalizability of results to all populations. The sites in West Africa and Indonesia diagnose and treat very large numbers of adults with active TB (500-1000 per year), and as a result see large numbers of family contacts. At present only very young children receive INH and the remainder are screened for active TB. But there is great interest in expanding this service - if feasible. If all sites enrol a total of 305 children annually (see section 2.15), we should complete enrolment within 3 years. As in the adult trial enrolment will be monitored closely, and corrective actions taken promptly at sites where enrolment is low.

2.13 ANTICIPATED PROBLEMS WITH COMPLIANCE AND TREATMENT COMPLETION

Adult

As discussed in Section 2.3, we expect differences in treatment completion to be the major determinant of differences in effectiveness of the two regimens. Our sample size calculations are based on these expected differences in completion rates, so recruitment will not be further increased to account for non-completion of therapy. We do not anticipate a major problem with "drop-ins" [166] during treatment i.e. patients who change therapy and cross over from one arm to the other, as this was seen in less than 1% of subjects randomized in Phase 2. Our planned secondary analysis of efficacy will be based on treatment completion, and assessment of compliance based on pill counting. This

measure correlated well with electronic monitoring in Phase 2, and with protective effect in prior trials [65].

Children:

We do not anticipate "problems" with compliance and treatment completion since this is a primary outcome of this study. If children change therapy and cross over from one arm to the other they will be considered to have reached an end-point of the study, and that they failed to complete the study drug arm to which they were randomized.

2.14 LOSS TO FOLLOW-UP

Our ability to follow all participants successfully for 28 months after randomization will be crucial. Our objective is to have less than 10% drop-outs; higher rates would be of concern, given the anticipated rate of the primary outcome of less than 3%. Loss to follow-up was 17% in Phase 2, although over 97% of subjects had some follow-up (see RCT Appendix 1: Table 7). Based on experience gained in Phase 2, we will ask for home, work, and cellular telephone numbers, plus email addresses of the study participants, at the time of their enrolment. We will also ask for four other contacts – close friends, or relatives living in the same city, or same country, or relatives remaining in their home country for recently arrived immigrants. This approach has been successful, in another ongoing trial of LTBI therapy at the Montreal Chest Institute, in keeping losses to less than 5% of those randomized, during 33 months of follow-up. The initial consent will include subjects' permission to verify their occurrence of active TB using local nominal TB reporting databases – for all sites, and provincial health administrative data-bases for Canadian sites. This will provide a mechanism (that we have used [181, 182]) to verify occurrence of active TB passively, and assess under-estimation of the study outcome, due to mortality or migration.

2.15 DESCRIPTION OF STUDY CENTRES, AND JUSTIFICATION FOR INTERNATIONAL SITES FOR ADULTS:

Selection of the international sites has been based on long-standing collaborative ties [172, 183-191], [192] between the site investigators and the PI. All involved countries have a substantial burden of TB, and are classified as having intermediate or °high incidence [2]. Inclusion of these sites strengthens the study for several reasons: (i) TB is a global disease that disproportionately affects low and middle income countries, where it is a very high priority health problem. (ii) LTBI therapy is under-utilized in these countries because it is viewed as impractical – but could have important benefits if therapy was simplified. (iii) Conduct of the trial in these international settings may help to demonstrate the feasibility, and cost-effectiveness of this strategy in these settings. This may enhance uptake of the findings in countries with a substantial TB burden. (iv) The training and conduct of this trial will strengthen capacity for clinical research in each country - an important long-term benefit. (v) These sites enhance the feasibility, and cost-effectiveness of the present trial.

Participating Canadian and international sites in Phase 3.(*Six will be new sites.**Brazil will double clinic sites)

	Number of patients treated per year (from 2005-2007)		Eligible per year, (estimated for new sites)	Annual enrolment	
	Active TB	LTBI		Phase 2	Projected
Montreal Chest	60-70	550-650	200	140	150
Saskatoon	80-90	300	50	25	30
Edmonton	70	450	100	60	75
Vancouver*	120	800	250-300	--	150
Rio de Janeiro, Brazil**	800	>1,000	>500	180	350
Riyadh, Saudi Arabia	100	300	150	70	70
Korea*	2560	na	2500	na	250
Australia*	300	123	120	na	75
Benin*	>1,000	na	500	na	250
Guinea*	>1,000	na	500	na	250
Ghana*	>1,000	na	500	na	100
Indonesia*	>1,000	na	500	na	250
TOTAL	>8,000	> 3,500	> 5,800	475	2000

BIOMARKER COMPONENT:

A few of the study sites for the parent trial will not participate (such as Benin, and Indonesia) because they lack adequate storage facilities to keep the QFT-GIT test supernatants frozen at -80C.

2.15.1 DESCRIPTION OF STUDY CENTRES, AND JUSTIFICATION FOR INTERNATIONAL SITES FOR CHILDREN: (REFER TO APPENDIX 11)

2.16 PRIMARY DATA ANALYSIS

Adult:

The primary study outcome will be the occurrence of microbiologically or histologically confirmed active TB, confirmed by the majority of the 3-member independent clinical review panel. The primary analysis, comparing the rate of occurrence of active TB per patient-year will be performed with the use of an unadjusted Poisson marginal model fitted using generalized estimating equations (GEE) to allow us to take clustering by household into account [193,194]. We will assume an exchangeable correlation structure. The log of follow up time will be used as an offset in the regression model, which will allow us to account for differing lengths of follow up time [194] If clustering is significant we will calculate a rate ratio from the GEE Poisson regression. But if the effect of clustering is negligible, as anticipated, the proportion of subjects randomized to both treatment groups developing confirmed active TB, and the associated 2-sided 95% confidence interval for the difference, will be estimated, using an incidence density method, expressed as TB events per 1000 person years of follow-up. This will allow us to include information from subjects who are followed for some time before being lost to follow-up.

Children:

Adverse events:

This outcome will be defined as the total of Grades 1-4 AE, that are diagnosed as probably related to the study drug by at least two of the three-member independent review panel. Following the method described by Kaul, we will declare 4RIF not inferior to 9INH in terms of AEs if the lower bound of the confidence interval around the difference in proportion of subjects experiencing an AE excludes the limit specified in section 2.11. The confidence interval will take potential clustering into account. Further descriptive analysis of AE including differences in type and severity of AE will be presented as exploratory, with appropriate caution. This analysis is justified by the very limited experience with 4RIF in children, making any information about tolerability and adverse effects of value.

2.16.1 Justification

Our planned primary analysis will be of study groups as randomized, i.e. an intention to treat analysis. This is recommended for superiority studies [166] because it provides a more conservative estimate of effect, since patients who did not take treatment per protocol are less likely to gain benefit. Hence differences between two regimens are attenuated making it more difficult to detect superiority.

2.16.2 DATA ANALYSIS FOR BIOMARKER COMPONENT

Planned analyses for the Biomarker component include comparison of change in T-cell cytokine response to TB specific antigens among all adult participants between those who did, or did not take at least 80% of planned doses 9 months after starting allocated LTBI therapy, and, among adult subjects who took at least 80% of doses, after 4 months between arms. For Objective 1, a linear regression model will look at the association between subjects compliant to RIF or INH treatment and change in T-cell cytokine level at 4 months, adjusting for potential confounders. This analysis will also allow adjustment for potential confounding by covariates including age, gender, indication for LTBI treatment, and center. Potential confounding may occur, because the comparison groups will not be those originally randomized, which may lead to imbalance between groups.

For Objective 2, a linear regression model will be fit to take advantage of the continuous nature of cytokine response and the continuous nature of pill taking behaviour, and allow adjustment for potential confounding by covariates (as listed above)

2.17 FREQUENCY OF INTERIM ANALYSIS AND STOPPING RULES FOR ADULT

2.17.1 Primary outcomes

Only one interim analysis of the primary outcome will be performed, one year after 33% of patients have been randomized. Further interim analyses, such as one year after 67% of subjects have been randomized, will fall too close to the end of randomization, to have any meaningful impact. We wish to avoid falsely concluding that one regimen has significantly superior effectiveness with this interim analysis – a well known risk [195,196]. Hence we will use a threshold of a p value <.001[196], before concluding that 4RIF is significantly **inferior** to 9INH , and stopping the trial early.

2.17.2 Serious adverse events (SAE)

Four months after randomization of 25%, 50%, and 75% of subjects, interim analyses will be performed of SAE. The DSMB will consider stopping the trial early if SAE rates are significantly ***higher*** with 4RIF. To balance the risk of unnecessarily stopping the study with interim analyses [196], with the need to ensure patient safety, we will use the method of Pocock [197], or an interim stopping level (p value) of 0.018 for each analysis.

2.17.2 FREQUENCY OF INTERIM ANALYSIS AND STOPPING RULES FOR CHILDREN (REFER TO APPENDIX 11)

2.18 PLANNED SUB-GROUP AND SECONDARY ANALYSES:

2.18.1 Active TB in subjects who complete treatment (efficacy)

Non-completion of therapy is obviously not a random event, and may be associated with characteristics that are associated with risk of disease[166]. Therefore we will compare the characteristics of compliant and noncompliant subjects in each group, and use logistic regression to estimate efficacy, adjusted for important covariates.

2.18.2 Confirmed and probable active TB

In secondary analysis we will combine these two outcomes, and use the same methods to compare rates of TB with both regimens, as described above for the primary analysis.

2.18.3 SAE

This outcome will be defined as the occurrence of Grade 3 or 4 SAE, diagnosed by the majority of the independent reviewers. Differences in all SAE, and by type of SAE between the two groups will be tested with Chi-squared or Fisher's exact tests. Sub-group analyses will compare rates of SAE by age, sex and HIV status. Drug interactions with 4RIF are common, and of particular interest since this will be the largest trial with mono-Rifampin therapy. All analysis of SAE and drug interactions will be presented as exploratory, with appropriate caution [166,196]. However in view of the very limited published experience with 4RIF, any information about adverse effects, including drug interactions, would be very useful for clinicians.

2.18.4 Drug resistance among cases of active TB after randomization

The occurrence of drug resistant TB after LTBI therapy may result from selection of drug resistant mutants during therapy (acquired resistance), or because they were infected with drug resistant organisms (primary resistance). All patients with positive cultures for M Tuberculosis within 28 months for adults and until 16 months for children after randomization will have drug sensitivity testing. Adequate facilities are available in all sites. If drug resistant tuberculosis is found, patients will be placed on appropriate therapy and followed by the site investigators in collaboration with the local TB programs. We will compare the prevalence of resistance to INH and RIF among study participants who develop active TB. The prevalence of underlying (or primary) INH resistance in the community will be assumed as the prevalence in those who took 4RIF, and of RIF resistance in those on 9INH. Acquired RIF resistance will be taken as the difference in prevalence of RIF resistance between subjects on 4RIF and 9INH. Power to detect significant differences will be limited, unless drug resistance is very common, but descriptive information on any excess resistance would still be useful.

2.18.5 PLANNED SECONDARY ANALYSES IN CHILDREN: (FOR DETAILS REFER TO APPENDIX 11)

Treatment completion, Active TB in subjetcs who complete treatment (efficacy) and Drug resistance among cases of active TB after randomization.

2.18.5 Cost-effectiveness of the two regimens

We will have detailed information on all health system activities – including scheduled visits, therapy and tests as part of routine follow-up, unscheduled visits, tests and therapy for adverse events, and diagnostic and therapeutic activities for the cases of active TB that develop. These will be valuated using local costs for all such activities (for details see RCT Appendix 4). We will also have measured incidence of active TB among subjects randomized to the two LTBI regimens. If one arm is cheaper and associated with fewer TB cases then it will be clearly preferable from a cost-effectiveness standpoint. If one arm is more expensive but associated with fewer TB cases then we will calculate the incremental cost per additional TB case prevented by the more effective (but more expensive) regimen. In primary analysis we will use average Canadian costs to valuate all health care activities, but in secondary analyses will perform the same analyses using costs from each site. This will be of particular interest in lower-income countries, where LTBI therapy is considered a low priority [158,199]. In sensitivity analyses we will vary the costs for active TB, given that the cases detected in this study will likely be at an early stage, so may underestimate average costs. We will also vary the costs of rifampin, which is extraordinarily expensive in Canada, compared to international prices[126].

2.19 RESULTS FROM PHASE 1 AND PHASE 2

Six years ago, we initiated a series of studies to evaluate 4RIF as therapy for LTBI. We felt the first essential requirement for 4RIF was that compliance and treatment completion had to be better than with 9INH. Hence these were primary end-points of the first study (Phase 1). Of 116 patients randomized equally to 4RIF or 9INH, 91% completed 4RIF with good compliance compared to 70% of those randomized to 9INH ($p<0.001$)[131 200] (See Research Module Appendix: Reprints).

The experience in 2000-2001 with 2RIF-PZA mandated a careful assessment of safety, conducted in Phase 2 - a multi-centre study primarily to compare serious adverse events (SAE) with 4RIF and 9INH. Consenting patients were randomized in equal numbers to 4RIF or 9INH using a web-based patient registration and randomization developed by Dr. Rousseau of the University of Sherbrooke. *To view the registration, randomization, and SAE reporting web-site, use Internet explorer (at least 6.0) to go to:* http://tbera-demo.crc.chus.qc.ca. *Username:* **dmenzies**, *password:* **review**; *domain:* **rsr** *(until Feb 1 2009). Enter these once when prompted, and then enter the same username and password a second time, when prompted again. This gives administrator rights to view a demonstration database, with fictitious patients.* Randomization was stratified by site in blocks of variable size. Investigators performed a standardized evaluation for each possible SAE. If the study drug was permanently discontinued, all clinical, lab, and follow-up information was reviewed independently by a three member panel, blinded to the study drug, to judge the type, severity and likely relationship to the study drug (Appendix 4).

We experienced more difficulties recruiting patients than anticipated at several Canadian sites. In the first year we intensified training and supervision, and opened a new sub-site. When this was not enough, investigator and CIHR approval was sought to shift budget allocations from low enrolling sites to start two new international sites – in Brazil and Saudi Arabia, ***within the originally awarded total budget***. Enrolment at all sites subsequently increased to average 40 patients per month. We had planned a sample size of 549 per arm to provide 80%

power (2 sided test) to demonstrate a significant difference in SAE rate if the true rate with 9INH was 4% and 4RIF was 9%, or was 5% with 9INH and 2% with 4RIF. After 25%, 50% and 75% of planned enrolment, the DSMB reviewed interim analyses – still blinded to the study drugs. In January 2007, the 3rd interim analysis revealed that SAE were significantly fewer in one arm. The DSMB asked to be unblinded, and when they learned that 4RIF had the lower rate of SAE, they recommended discontinuation of enrolment. Of 420 subjects randomized to 4RIF, 7 developed Grade 3-4 adverse events attributed to study therapy by the independent panel, compared to 17 of the 427 on 9INH (Risk difference (4RIF-9INH): -2.3%; [95% Confidence interval: -0.1% to -5%] p=.04). Grade 3-4 hepatitis occurred in 3 taking 4RIF, compared to 16 who started 9INH (-3.1%; [-1% to -5%], p=.003). Grade 1 or 2 adverse events attributed to study drugs were similar in the two arms. Asymptomatic reduction in platelet count and white blood count were significantly more frequent during treatment with 4RIF. Completion rates were 78% with 4RIF and 60% with 9INH (Risk difference: 18% [12% to 24%], p<.001) [148]. Average health system costs were significantly lower with 4RIF. Incremental cost effectiveness analysis revealed that 4RIF would be cost saving and prevent more cases if efficacy was at least 75%, and would be cost saving if efficacy is more than 65% [147].

We believe that a study to assess effectiveness of 4RIF is now justified. This regimen has been demonstrated to have better completion, lower costs, and is safer than 9INH, particularly for hepatotoxicity - the most important and potentially lethal adverse event of INH therapy (and of 2RIF-PZA). In Phase 2, 847 subjects were enrolled, randomized, treated and followed to detect active TB - using the methods proposed here, making the present proposal more cost-effective.

SECTION 3 TRIAL MANAGEMENT

3.1 DAY TO DAY RUNNING (See also RCT Appendix 7 - Time-Table)

This is described in Sections 2.3 (recruitment), 2.7 (follow-up/data gathering), and 2.8-2.9 (measuring outcomes).

3.2 ROLE OF APPLICANTS

Dr Menzies will chair the trial steering committee, supervise the central coordinating staff, liaison with site PI's, and take primary responsibility for overall data analysis and report writing. Each site PI will supervise all aspects of site trial management (ethics, recruitment, and follow-up), and site-specific staff. To take advantage of members' expertise, added specific responsibilities will be assigned as described in detail in RCT Appendix 6.

3.3 TRIAL STEERING COMMITTEE, DSMB, AND CLINICAL REVIEW PANEL

The trial steering committee will consist of the PI, site investigators, and study manager (Mme Hornby). They will meet by telephone conference every 3 months in the first year, and then every 6 months, to review recruitment, randomization, operational issues, and DSMB reports. This committee will decide on early termination (if recommended by the DSMB), major protocol and/or consent modifications, and budget re-allocations. The membership, responsibilities, and functioning of the DSMB are described in Section 2.9.3, and of the clinical review panel in Section 2.9.1.

References for children: refer to appendix 11

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SUMMARY OF CHANGES IN STUDY PROTOCOL

Randomized trial to compare 4 months Rifampin with 9 months INH – History of changes/amendments:

Version 1: 4v9Adult: Randomized trial to compare 4RIF with 9INH for Latent TB: Phase 3 - effectiveness
Version 2 – amendment to change some eligibility criteria, practice and/or authoritative guidelines, pragmatic change
Version 3 – amendment to add Biomarker pilot study – described in appendix 10 (mention at point 2.18.6)
Version 4 – amendment to add Biomarker full study - described in new appendix 10
Version 5 – amendment to add Pediatric study (for sites who did adult and peds, but not Biomarker) - described in appendix 11
Version 6 – amendment to add Pediatric and biomarker full study

SUMMARY OF CHANGES:
Version 2 (changes from Version 1):

Error in original protocol: TST conversion – there was an error in the original protocol and the definition of TST conversion for someone who has documented baseline negative test between 2 and 5 years ago should be a "TST increase by 10mm with final TST >10 mm. This has been corrected.

CHANGES:
1. *Current protocol*: eligible if TST $\geq$ 10mm, and to start TNFa inhibitors
Proposed change: eligible if TST $\geq$ 5mm, and to start TNFa inhibitors, or on transplant anti-rejection medications.
Rationale: This change would bring our criteria in line with current ATS/CDC recommendations for this group of patients.

2. *Current protocol*: eligible if TST positive (risk stratified 5/10/15mm). If Quantiferon (QFT) done the QFT results are not considered (ie QFT could be positive or negative) as long as TST fits risk stratified criteria and MD recommends LTBI therapy.
Proposed change: Eligible if TST is not done but QFT (or T-Spot) done and positive (according to manufacturers recommendations). A positive QFT will be considered equivalent to a TST of 10 mm at the time of randomization (ie not an independent risk factor like the TST of 15 mm).
Rationale: Increasingly at some study sites (and many non-study sites) providers are only doing QFT. If QFT is positive, the providers and the patients do NOT want to do a TST. The TST could cause a strong reaction, such as blistering. As well performance of the TST would cause delays and patients would need to make at least one more visit before treatment is started. And, perhaps most importantly, the TST results would not change the providers' decision to treat.

3. *Current protocol*: Eligible if < 90% Ideal body weight (this is a minor criterion that requires the presence at least one other criterion for patients to be eligible).
Proposed change: Eligible if BMI <19
Rationale: This is more easily measured and calculated, and more standardized for all populations.

4. *Current protocol*: Photos are taken of skin rashes, to allow more objective judging of these allergic adverse events.
Proposed change: Drop this and rely on a careful description of the rash.
Rationale: In phase 2 very few patients were adequately photographed at the time of the rash and then again after resolution. Therefore the DSMB who reviewed these cases did not find photos helpful at all. Instead they relied upon simple narrative description. In Phase 3 we plan to detect rashes using narrative description only. As well, in phase 3

we will have a substantial number of participants who are black. In these patients, the visual appearance of a rash is very difficult to discern, and photos are almost useless. Accordingly we plan to drop the photos of rashes from the protocol and the consent.

5. *Current protocol:* Patients excluded if on certain anti-retroviral therapy (ART); certain currently used ART drugs are not on our list.
Proposed change: Update annually to be concordant with latest CDC guidelines, and reflecting latest drugs that are in clinical use.
Rationale: Up-dated CDC guidelines reflect current practice better. (This is not really a protocol change but rather is just part of our SOP).

6. *Current protocol*: Minimum follow-up of patients on therapy is monthly for first 4 months then as per provider, but at least every 2 months.
Proposed change: Minimum will be monthly for first 2 months, then minimum every 2 months thereafter.
Rationale: Many sites are finding the frequency of follow-up exceeds their usual standard of care. This tends to inflate costs and discourage completion of therapy, especially for INH since total visits will typically be 6-7 (hence the current protocol may actually introduce bias in favour of 4RIF).

7. Minor corrections to protocol:
(i) TST conversion – the current protocol contains an error. The definition of TST conversion for someone who has documented baseline negative test between 2 and 5 years ago should be a "TST increase by 10mm with final TST $\geq$10 mm".

(ii) The minor eligibility criteria should read 2 of the following 4 factors (not 5 factors as printed now. There are only 4 factors listed)

Version 3 (changes from Version 2): Pilot Biomarker study added

Pilot study for Biomarkers added. This was initiated at a single site while awaiting funding. All the relevant changes were taken from the biomarker study protocol (submitted for funding) and inserted in the text of the main protocol. Appendix 10 was added – summarizing information for the investigators.
No actual changes to study eligibility, enrolment, randomization, treatment, or follow-up. Simply added blood samples drawn before initiation of therapy, at 1 month after commencement of therapy, and at the end of LTBI treatment. Samples frozen for later analysis after the study completed.

Version 4 (changes from Version 2): Full Biomarker study added

Funding awarded from CIHR for Biomarker study, so Biomarker study expanded to all participating sites. Relevant changes taken from grant proposal and inserted into V4.

No changes to study eligibility, enrolment, randomization, treatment, or follow-up. Only change is added blood samples drawn at pre-treatment, and 4 and 9 months after randomization. Samples frozen for later analysis after the study completed.

Version 5 (changes from Version 2): Pediatric study added (A randomized trial to compare completion and tolerability of 4 months Rifampin (4 RIF) and 9 months Isoniazid (9 INH) for treatment of latent TB in children). Funding awarded from CIHR, so study initiated in children. Appendix 11 was added – summarizing information for the investigators.
No change in Adults except increase in sample size to 6,000 total to be enrolled in Phase 3 due to greater loss to follow-up in Phase 2 (at that time Phase 2 follow-up completed).

OBJECTIVES

Current – Adult: - (Primary) Compare the effectiveness and (secondary) compare completion safety and tolerability.

Children: - Compare safety, tolerability (primary) and completion rates (secondary).

TRIAL DESIGN

Current- Adult: Study was done only on adults subjects (over ≥18 yrs old).

Children: For children up to and including 17 years of Age.

Current – Adult: We need to recruit a total of 6000 subjects.

Children: Need to Recruit 822 children in addition to adult target.

Current – Adult: Followed up to 28 months after randomization.

Children: Followed up to 16 months after randomization.

INTERVENTION

Current- Adult: Dose given: 9 months INH - 5 mg/kg/day (max=300mg/day) and 4 months RIF - 10 mg/kg/day for adults. Dosage will be adjusted if weight is less than 42 kg at 200mg/day.

Children: Dose given will be: 9 months INH - 10-15 mg/kg/day (max=300mg/day) and 4RIF - 10-20 mg/kg/day (max=600mg/day). Dosing for both INH and RIF will be age and weight dependant, with highest doses for infants, and lowest for adolescents.

Drug doses are in accordance with the newly revised WHO pediatric recommendations (INH 10-15mg and RIF 10-20mg) that were compiled following a comprehensive review of the existing literature. No child will exceed the maximum mg/kg dose advised by WHO, while the dose range will be controlled within a narrow 5mg/kg band for both drugs.

Recommended Doses by Weight for latent TB therapy in children

Isoniazid (INH) (liquid suspension 10mg/ml)
Rifampin (Rif) (liquid suspension 10mg/ml)
Children above 15kg may take tablets – alone or in combination with syrup

Weight (kg)	INH dose 10-15mg/kg	RIF dose 15-20mg/kg
3.1- 4	40	60
4.1- 5	50	75
5.1- 6	60	90
6.1-8	80	120
8.1-10	100	150
10.1-12	120	180
12.1-15	150	225
15.1-20	200	300
20.1-25	250	400
25.1-30	300	500
30.1-35	300 (maximum)	600 (maximum)

PROCEDURES OF RECRUITMENT

Current- Adult: Informed consent form.

Children: Informed assent and parental consent form.

PROTECTION AGAINST BIAS

PREVENTING BIAS IN ASCERTAINMENT OF THE PRIMARY OUTCOME

Current-Adult: Active TB-reviewed by 3 independent members of clinical panel, blinded to the study drug

Children: Adverse Events - A 3-member independent panel will review each event, blinded to the study drug, and opinions of the other panel members to judge relationship to study drug, and grade severity.

PREVENTING BIAS IN ASCERTAINMENT SECONDARY OUTCOME

Current-Adult: Adverse events-reviewed by 3 independent members of clinical panel, blinded to the study drug.

Children: Completion of treatment count - based on dosage.

Active TB - An independent 2-member clinical panel (Drs Marais and Schaaf of Stellenbosch Univ, Capetown) will review all clinical, radiologic, and lab information. Both are paediatricians with internationally recognized expertise in diagnosis and management of paediatric TB; neither are investigators at enrolling sites. Panel members will independently judge each case as confirmed, probable, or unlikely TB,

INCLUSION CRITERIA

Current adult:

Adults (age ≥ 18) with documented positive TST as defined below and prescribed 9INH for LTBI:

Note: In the absence of a TST test, a positive QFT (or T-Spot) is equivalent to a TST of 10 mm.

- HIV positive, OR to start TNFa inhibitors, OR on transplant anti-rejection medications. (TST ≥5 mm or QFT +)
- Close contact: ≥4 hours contact per week, for ≥ 1 week with person with active pulmonary TB. (TST ≥5 mm or QFT +)
- Apical/upper lobe fibronodular disease with area >2cm^2 (TST ≥5 mm or QFT +)
- Documented tuberculin conversion within two years. (Increase ≥ 6 mm, with subsequent TST ≥10 mm or QFT +)
- Diabetes, renal failure, or immuno-compromised from medical condition or therapy (TST ≥10 mm or QFT +)
- Casual contact: contact of <4 hours/week, with a person with smear positive pulmonary TB. (TST ≥10 mm or QFT +)
- Tuberculin conversion within 2-5 years. (Increase of 10 mm or more with subsequent TST ≥10 mm or QFT +)

Have (1) **TWO** of the following **four** factors if TST = 10-14mm or QFT +,

OR (2) **ONE** factor if TST ≥15mm:

e. Arrival in Canada, Australia, or Saudi Arabia in the past 2 years from countries with WHO estimated incidence greater than 100 per 100,000. Or indigenous Canadian living on reserve.

f. BMI <19;

g. Any abnormality on chest x-ray compatible with past-TB infection e.g. calcified granuloma, or hilar lymph nodes, costo-phrenic angle blunting - other than fibronodular disease above.

h. Cigarette smoking (at least a half pack per day) currently.

Children:

Children (age <18) with documented positive TST as defined below and prescribed 9INH for LTBI, for the indications below,

- HIV positive (TST ≥5 mm or QFT +).
- Age 5 or less (TST ≥5 mm or QFT +).
- Other reason for immuno-compromised state - such as therapy for malignancy or post-transplant (TST ≥5 mm or QFT +).
- Contact: with adult or adolescent with active contagious pulmonary TB. (TST ≥5 mm or QFT +)

Have **both** of the following factors if TST = 10-14mm or QFT +, or **one** factor if TST ≥15mm:

- Arrival in Canada, Australia, or Saudi Arabia in the past 2 years from countries with estimated annual incidence of active TB greater than 100 per 100,000. Or indigenous Canadian living on reserve.
- Body mass index (BMI) less than 10th percentile for their age.

The TST may be negative for up to 8 weeks after primary infection, before adequate cell mediated immunity develops. Because of this, current practice is to begin LTBI treatment therapy immediately for children ≤ 5 years old, even if TST negative. After 8-10 weeks the TST is repeated; LTBI therapy is continued if now TST positive, and stopped if TST still negative.

Children maybe enrolled if contacts and initial TST negative. If TST negative after 8-10 weeks they will be excluded from the analysis of treatment completion, but included in the incidence density analysis (person-time) of tolerability and safety.

EXCLUSION CRITERIA

Current adult:

1. Patients who were contacts of TB cases known to be resistant to INH, RIF, or both (i.e. MDR).
2. Known HIV-infected individuals on anti-retroviral agents whose efficacy would be substantially reduced by Rifampin, unless therapy can safely be changed to agents not affected by Rifampin (listed in RCT quick guide - Appendix 5).
3. Pregnant women - Rifampin and INH are considered safe in pregnancy, but therapy is usually deferred until 2-3 months post-partum to avoid fetal risk and the potential for increased hepato-toxicity immediately post-partum.
4. Patient on any medication with clinically important drug interactions with INH or RIF, which their physician believes would make either arm contra-indicated. An updated list of clinically important drug interactions is in the RCT Quick Guide (Appendix 5). This includes women taking hormonal contraceptives who will not take alternative contraception.
5. History of allergy/hypersensitivity to Isoniazid or to Rifampin, Rifabutin or Rifapentine.
6. Active TB. Patients initially suspected to have active TB can be randomized once this has been excluded.

Children:

Criteria 1 to 6 are the same as for adults.

Criteria 7- Prior complete LTBI therapy or if children have taken >1 week and are still taking the treatment. Children will be eligible if they took an incomplete LTBI therapy (less than 80% of recommended total dose) but > 6 months ago.

FOLLOW-UP AND DATA GATHERING

INITIAL EVALUATION

Current adult: HIV test will be offered to all adult subjects.

Children: HIV test will not be offered except if clinically indicated (Risk factor for HIV in the children)

FOLLOW-UP DURING TREATMENT

Current adult: Blood test (CBC and liver transaminases) done at pre-treatment and at the first follow-up visit.

Children: Blood test (CBC and liver transaminases) done at pre-treatment only, unless symptoms or problem arise during follow-up.

THE PRIMARY AND SECONDARY OUTCOME

Current adult: (Primary) Active TB and (secondary) Compliance, SAE, Costs, and Drug resistance.

Children: (Primary) Intolerability/Safety - AE and (secondary) Treatment completion, Active TB, and Drug resistance.

PRIMARY AND SECONDARY ANALYSES

Current adult: (Primary) Comparing the rate of occurrence of Active TB
(Secondary) Active TB in subjects who complete treatment (efficacy),
Confirmed and probable active TB, SAE and,
Drug resistant among cases of active TB after randomization.

Children: (Primary) AE grade 1-4
(Secondary) Treatment completion,
Active TB in subjects who complete treatment (efficacy) and,
Drug resistance.

Interim analyses: Two interim analyses will be done: At 4 months after enrolment of 25% and 50% of subjects – (this allows for completion of RIF). We will compare serious adverse events by study arms. This report will go to the DSMB for review. They will be blinded to study drug (i.e. Drug A and B). Stopping rules: if the group being treated with RIF have significantly greater numbers of serious adverse events than those being treated with INH the study will be stopped for children. To account for multiple testing will use Bonferoni correction for multi testing – and so the P value will have to be <.015 (ie RIF SAE > INH with P<.015).

Version 6 (changes from Version 5): Biomarker study added:

All sites initially agreed to enrol adults for the Phase 3 trial, and obtained ethical approval for this trial. When the Biomarker and Pediatric sub-studies were added, some sites could enrol children, and some could not. Other sites agreed to participate in the Biomarker study, but the requirement to ship samples to the coordinating centre (Montreal) meant that certain international sites could not participate due to national regulations concerning shipment of biological samples out of these countries. Hence 3 versions of the protocol (Version 4, 5 and 6) were required – depending on which of the two sub-studies each site was participating in – Version 6 was for sites participating in both.

However, there were no further changes from Version 4 (adult with Biomarker) or Version 5 (adult with pediatric). These changes are described above.