AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Efficacy and safety of high-dose rifampin in pulmonary tuberculosis: a randomized controlled trial

Authors
Gustavo E. Velásquez,[1,2] Meredith B. Brooks,[2] Julia M. Coit,[2] Henry Pertinez,[3] Dante Vargas Vásquez,[4] Epifanio Sánchez Garavito,[5] Roger I. Calderón,[6] Judith Jiménez,[6] Karen Tintaya,[6] Charles A. Peloquin,[7] Elna Osso,[2] Dylan B. Tierney,[8] Kwonjune J. Seung,[8,9] Leonid Lecca,[2,6] Geraint R. Davies,[3]* Carole D. Mitnick[2,8,9]*

Affiliations
[1] Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA, USA
[2] Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, USA
[3] Institutes of Infection and Global Health and Translational Medicine, University of Liverpool, Liverpool, UK
[4] Hospital Nacional Hipólito Unanue, Lima, Peru
[5] Hospital Nacional Sergio Bernales, Lima, Peru
[6] Partners In Health / Socios En Salud Sucursal Peru, Lima, Peru
[7] College of Pharmacy and Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
[8] Division of Global Health Equity, Brigham and Women's Hospital, Boston, MA, USA
[9] Partners In Health, Boston, MA, USA
* Co-senior authors

The project described was supported by the National Institute of Allergy and Infectious Diseases (NIH/NIAID grants U01 AI091429 to C.D.M. and G.R.D., L30 AI120170 and UM1 AI068636 to G.E.V.). G.E.V. received support from the Ronda Stryker and William Johnston Fellowship in Global Health and Social Medicine and the Dr. Lynne Reid/Drs. Eleanor and Miles Shore Fellowship at Harvard Medical School, the Burke Global Health Fellowship at the Harvard Global Health Institute, and the AIDS Clinical Trials Group Minority HIV Investigator Mentoring Program. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or the institutions with which the authors are affiliated. The funding source had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Author contributions: G.R.D. and C.D.M. designed the study. G.E.V., M.B.B., G.R.D., and C.D.M. interpreted the data and wrote the first draft of the manuscript. M.B.B., J.M.C., D.V.V., E.S.G., R.I.C., J.J., K.T., C.A.P., E.O., L.W.L., G.R.D., and C.D.M. implemented the study. D.V.V., E.S.G., R.I.C., J.J., K.T., and C.A.P. collected the data. G.E.V., M.B.B., H.P., and G.R.D. performed the analysis. J.M.C. assisted with data management. All authors revised the manuscript critically for important intellectual content and gave final approval of the version to be published.

Correspondence should be addressed to Gustavo E. Velásquez, MD MPH, Department of Global Health and Social Medicine, Harvard Medical School, 641 Huntington Avenue, Boston, MA

02115. Telephone: +1.617.432.1707; Fax: +1.617.432.2565; Email: gvelasquez{at}bwh.harvard.edu

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

At a Glance Commentary:

Scientific Knowledge on the Subject

The standard of care for patients with new pulmonary tuberculosis is a six-month, four-drug regimen that includes rifampin for the full course of therapy. Attempts to further shorten tuberculosis therapy in the 1970s with higher, intermittent doses of rifampin were unsuccessful due to an apparent increase in toxicity. Renewed interest in tuberculosis treatment shortening developed earlier this century, with trials of fluoroquinolones, rifapentine, and a shortened regimen in patients at lower risk for poor outcomes. We sought to systematically examine the concept that increased daily doses of rifampin could shorten standard therapy for tuberculosis and improve treatment outcomes without increased toxicity.

What This Study Adds to the Field

This blinded, randomized, controlled Phase II clinical trial assessed differences across three daily oral doses of rifampin (10, 15, and 20 mg/kg) in change in elimination rate of *Mycobacterium tuberculosis* in sputum and frequency of rifampin-related adverse events. We found that doses of 15 and 20 mg/kg/day resulted in more rapid change in counts of *M. tuberculosis* and a similar frequency of rifampin-related adverse events. This is the first controlled study to show both dose-and exposure-response of rifampin on sputum sterilization. Our findings support the continued investigation of higher doses of rifampin, beyond 20 mg/kg, for potential treatment shortening.

Running title: High-dose rifampin for pulmonary tuberculosis

Descriptor number: 11.6 (Treatment of Tuberculosis or Latent Infection)

Total word count (excluding abstract, references, legends; max 3,500): 3,500

At a Glance word count (max 200): 200

Abstract

Rationale: We examined whether increased rifampin doses could shorten standard therapy for tuberculosis without increased toxicity.

Objectives: To assess the differences across three daily oral doses of rifampin in change in elimination rate of *Mycobacterium tuberculosis* in sputum and frequency of rifampin-related adverse events.

Methods: We conducted a blinded, randomized, controlled Phase II clinical trial of 180 adults with new smear-positive pulmonary tuberculosis, susceptible to isoniazid and rifampin. We randomized 1:1:1 to 10, 15, and 20 mg/kg/day of rifampin during the intensive phase. We report the primary efficacy and safety endpoints: change in elimination rate of *M. tuberculosis* $\log_{10}$ colony forming units and frequency of grade 2 or higher rifampin-related adverse events. We report efficacy by treatment arm and by primary (AUC/MIC) and secondary (AUC) pharmacokinetic exposure.

Measurements and Main Results: Each 5 mg/kg/day increase in rifampin dose resulted in differences of -0.011 (95%CI, -0.025 – +0.002;*P*=0.230) and -0.022 (95%CI, -0.046 – -0.002;*P*=0.022) $\log_{10}$ colony forming units/mL/day in the modified intention-to-treat and per-protocol analyses, respectively. Elimination rate in the per-protocol population increased significantly with rifampin $AUC_{0\text{-}6}$ (*P*=0.011) but not with $AUC_{0\text{-}6}/MIC_{99.9}$ (*P*=0.053). Grade 2 or higher rifampin-related adverse events occurred with similar frequency across the three treatment arms: 26(43.3%), 31(51.7%), and 23(38.3%) participants had at least one event (*P*=0.7092) up to 4 weeks after the intensive phase. Treatment failed or disease recurred in 11(6.1%) participants.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Conclusions: Our findings of more rapid sputum sterilization and similar toxicity with higher rifampin doses support investigation of increased rifampin doses to shorten tuberculosis treatment.

Abstract word count (max 250): 250

Keywords: tuberculosis, rifampin, randomized controlled trial, treatment efficacy, adverse drug event

Introduction

Tuberculosis is the leading infectious cause of death worldwide, killing 1.7 million in 2016 (1). Standard treatment for the annual estimated 10.4 million patients with new pulmonary tuberculosis is a six-month, four-drug regimen including rifampin throughout (2).

Combining rifampin and pyrazinamide in the early 1970s shortened tuberculosis treatment from 18 to 6 months (3). Further attempts to shorten tuberculosis therapy in the 1970s were guided by perceived cost constraints: any increase of the dose was matched by decreased frequency of administration. The resulting higher, intermittent doses of rifampin led to increased toxicity (4-6). Ultimately, the 600 mg daily dose of rifampin was selected as the standard (7). Renewed interest in tuberculosis treatment shortening developed over the last 20 years, with preclinical and clinical studies of fluoroquinolones and one trial of a further-shortened regimen in patients with noncavitary disease and rapid bacteriological response (8-14). Prior to the initiation of the present study, a Phase II trial of treatment shortening with another rifamycin, rifapentine, was published; further work on treatment shortening with rifapentine was subsequently pursued (15-17).

Daily dose optimization of rifampin remained a priority. Unlike moxifloxacin and rifapentine, rifampin is an established first-line drug, available throughout the world, from multiple suppliers, now for pennies a capsule. It is also available in combination formulations. Of the four current first-line agents, rifampin has the most potent sterilizing activity and has been established as key to the continuation phase of treatment (18, 19). At the 600 mg daily dose, rifampin is well-

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

tolerated (20-23). However, concern about the toxicity of rifampin at higher doses persists pursuant to trials of intermittent dosing (6, 20, 24).

While multiple controlled trials began to evaluate higher rifampin doses in African populations (25-27), and a single observational study evaluated rifampin concentrations in routine conditions in Peru (28), none evaluated, under controlled conditions, the concentration of rifampin and its concentration-dependent activity in Latin American patients. Prior evidence indicates important population variability in these measures for antituberculous drugs (29-32). No other late-stage clinical study has explored efficacy as a function of the pharmacokinetic/pharmacodynamic parameter thought to best predict rifampin efficacy (AUC/MIC) (22, 33-35). The present clinical trial entitled "Evaluation of high-dose rifampin in patients with new, smear-positive tuberculosis" (HIRIF) was designed to examine the concept that increased rifampin doses could shorten standard therapy for tuberculosis and improve treatment outcomes without increased toxicity. The primary objectives of this study were to assess the differences across three daily oral doses of rifampin (10, 15, and 20 mg/kg) in: plasma concentrations of rifampin; change in elimination rate of *Mycobacterium tuberculosis* in sputum; and frequency of grade 2 or higher adverse events (AEs) related to rifampin. Here we report the primary efficacy and safety endpoints. The pharmacokinetic results have been previously published (36), and some of the results of this study have been previously reported in the form of abstracts (37-39).

Methods

Design

HIRIF was a blinded, randomized, controlled Phase II clinical trial registered under an investigational new drug application with the U.S. Food and Drug Administration (106635) and with ClinicalTrials.gov (NCT01408914) investigating the pharmacokinetics, efficacy, and safety of higher doses of rifampin in patients with pulmonary tuberculosis. Participants were identified and referred for enrollment from health centers in Lima, Peru between September 2013 and February 2015. Eligible participants were previously untreated adults (18-60 years old) with smear-positive disease (≥2+) and strains susceptible to isoniazid and rifampin. Exclusion criteria included a contraindication to rifampin and certain extrapulmonary manifestations of tuberculosis. All participants provided written informed consent. Additional details on study methods are provided in the published protocol (40) and online supplement.

Intervention

Participants were randomly allocated (1:1:1) in blocks of varying size to receive 10, 15, or 20 mg/kg/day of rifampin during the 8-week intensive phase (see Table E1 for dosing by weight band). The randomization sequence was generated by the unblinded study statistician and programmed into the electronic data capture (EDC) system. All other study staff (including laboratory staff) were blinded, except pharmacy staff who received the arm assignment from the EDC and prepared study-drug kits. Standard doses of rifampin and companion drugs (isoniazid, pyrazinamide, ethambutol) were delivered through fixed-dose combinations (FDCs) supplied by MacLeods Pharmaceuticals (Mumbai, India) (2). These were supplemented by 150 mg capsules of rifampin and matched placebo donated by Sanofi (Paris, France). During the 18-week

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

continuation phase, participants received isoniazid supplied by Micro Labs Limited (Bangalore, India) and rifampin, both dosed at 10 mg/kg thrice weekly. Participants received pyridoxine 50 mg thrice weekly throughout treatment. Treatment was ambulatory and directly observed.

Protocol-defined safety halts occurred when rifampin-related serious AEs met prespecified halting criteria (Table E2) (40). During safety halts, all subjects were treated with a standard regimen according to local guidelines (with an intensive phase rifampin dose of 10 mg/kg/day). When the sponsor and the data safety and monitoring board determined that the study could resume, participants in the intensive phase of treatment reinitiated their assigned experimental doses.

Outcomes

The primary pharmacokinetic/pharmacodynamic (PK/PD) endpoint, area under the concentration-time curve (AUC) of rifampin in plasma at steady state divided by the minimum inhibitory concentration of 99.9% of *M. tuberculosis* ($MIC_{99.9}$), has been previously reported (36). The primary efficacy endpoint was the change in elimination rate of *M. tuberculosis* $\log_{10}$ colony forming units (CFU) per mL in overnight pooled sputum samples collected throughout the intensive phase and cultured on 7H11 Middlebrook medium. Secondary efficacy endpoints included proportion of culture conversion in Löwenstein-Jensen (LJ) medium at 8 weeks, and proportion of unfavorable outcomes (treatment failure, recurrence after cure, or death) at 12 months. We classified recurrent disease with identical 24-locus mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU-VNTR) sequencing as relapsed disease, and nonidentical MIRU-VNTR as reinfection. The primary safety endpoint was the frequency of

grade 2 or higher rifampin-related AEs, per the Division of Microbiology and Infectious Diseases' Adult Toxicity Table (41), during the intensive phase and up to 4 weeks thereafter.

Statistical Analysis

We defined the intention-to-treat (ITT) population as all participants who received at least one dose of study medication. The modified intention-to-treat (mITT) population (prespecified as primary for efficacy) included those in the ITT population with more than one non-missing, detectable, and plausible quantitative culture result. The per-protocol (PP) population included those in the mITT population whose experimental rifampin dose was not affected by study halts. Sensitivity analyses were performed on all participants with at least one $\log_{10}$CFU/mL count. We analyzed $\log_{10}$CFU/mL using linear and nonlinear mixed effects models in NONMEM 7.2 (ICON plc, Dublin, Ireland), Perl-speaks-NONMEM 4.4.0 (Uppsala University, Uppsala, Sweden) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Monophasic and biphasic structural models were fitted, by treatment arm and pharmacokinetic exposure, using the M3 partial likelihood method and Laplacian estimation with interaction.

We used the one-sided Cochran-Armitage test for trend with 5% significance to compare proportions of participants who experienced at least one grade 2 or higher rifampin-related AE. We used the log-rank test to compare the time to AEs. We performed similar tests for secondary analyses of rifampin-related hepatic AEs and serious AEs (SAEs). A sensitivity analysis excluded AEs that occurred during study halts in the 15 and 20 mg/kg arms. We performed all safety analyses in Stata/SE 14.2 (StataCorp LLC, College Station, TX).

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Results

We screened 351 patients for eligibility; of these, 180 were randomized to receive study drug, 60 to each arm (Figure 1). Three study halts occurred during the study. The data safety and monitoring board recommended restarting enrollment and study dosing after each of the halts. Details on the study halts are provided in Table E2. The trial ended when all enrolled participants completed follow-up.

The mITT dose-efficacy analysis included 174 participants. Six participants were excluded for inadequate data: four had only one CFU observation; one had all CFU observations below the limit of detection (<10 CFU/mL); and one had implausibly discrepant CFUs in two samples obtained 9 days apart. The PP dose-efficacy analysis included 132 patients in the mITT population whose intensive phase rifampin dosing was unaffected by study halts. The mITT and PP exposure-efficacy analyses included 163 and 126 patients from the respective dose-efficacy populations who completed a PK sampling visit (Figure 1). All 180 participants were included in the safety analysis. Most participants were young (median age 25) and male (63.3%). Median weight was slightly higher in the 15 mg/kg arm, 55.4 kg (interquartile range [IQR], 49.2-60.5 kg) compared to 53.5 kg (IQR, 50.6-59.6 kg) and 54.9 kg (IQR, 50.3-61.7 kg) in the 10 and 20 mg/kg arms, respectively. Over half of participants had a baseline smear grade of 3+ (51.7%); slightly more patients in the 15 mg/kg arm (60.0%) than in the 10 mg/kg (46.7%) and in the 20 mg/kg (48.3%) arms had a 3+ smear grade. The mean baseline mycobacterial load was 5.2 $\log_{10}$CFU/mL. The mean baseline loads were 4.8 and 5.5 $\log_{10}$CFU/mL among participants with 2+ and 3+ smear grades, respectively. Cavitary disease was present in 66 (37.1%). HIV coinfection and diabetes were rare, present in 5 (2.8%) and 1 (0.6%) participants respectively.

Other than weight and smear grade, the distribution of sociodemographic characteristics, comorbidities, clinical indicators of disease severity, and mycobacterial load were similar across arms. Participants received median rifampin doses of 450 mg (IQR, 450-600 mg), 900 mg (IQR, 600-900 mg), and 975 mg (IQR, 750-1200 mg) in the 10, 15, and 20 mg/kg arms, respectively (Table 1).

Efficacy

Efficacy outcomes are shown in Tables 2 and 3, and Figure 2. A monophasic model of bacterial elimination best described the data. We estimated the difference in elimination rate between the treatment arms as -0.011 (95% confidence interval [CI], -0.025 – 0.002) $\log_{10}$CFU/mL/day for each 5 mg/kg increase in rifampin dose in the mITT population (P=0.230). In the PP population, the difference was -0.022 (95% CI, -0.046 – -0.002; P=0.022). Elimination rate in the mITT population did not increase with plasma rifampin $AUC_{0\text{-}6}$ (-0.001 $\log_{10}$CFU/mL/day per 1 log increase in $AUC_{0\text{-}6}$; 95% CI, -0.003 – 0.001; P=0.750) or $AUC_{0\text{-}6}/MIC_{99.9}$ (-0.002 $\log_{10}$CFU/mL/day per 1 log increase in $AUC_{0\text{-}6}/MIC_{99.9}$; 95% CI, -0.005 – 0.002; P=0.330). Elimination rate in the PP population increased with plasma rifampin $AUC_{0\text{-}6}$ (-0.017 $\log_{10}$CFU/mL/day per 1 log increase in $AUC_{0\text{-}6}$; 95% CI, -0.029 – -0.007; P=0.011), but not with $AUC_{0\text{-}6}/MIC_{99.9}$ (-0.010 $\log_{10}$CFU/mL/day per 1 log increase in $AUC_{0\text{-}6}/MIC_{99.9}$; 95% CI, -0.021 – 0.000; P=0.053). These results were not affected by adjustment for important baseline covariates including age, sex, and extent of radiological disease. The proportion of participants with 8-week culture conversion in LJ medium was nearly identical across the three treatment arms (76.7%, 73.3%, and 75.0%, respectively). Eleven (6.1%) participants experienced unfavorable outcomes by the end of the 12-month follow-up (7.5% of the 147 participants in

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

whom 12-month status could be assessed). Five (2.8%) participants experienced treatment failure. Of these, three (60%) were in the 10 mg/kg arm. Six (3.3%) more experienced recurrent disease, three (50%) in the 10 mg/kg arm; two (33%) were confirmed relapses by MIRU-VNTR sequencing. There were no deaths during the study. A detailed description of all treatment failure and recurrence outcomes is provided in Table E3.

Safety

The frequency of grade 2 or higher rifampin-related AEs was similar across treatment arms: 26 (43.3%), 31 (51.7%), and 23 (38.3%) of participants in the 10, 15, and 20 mg/kg arms respectively had at least one event (P=0.7092) (Tables 4 and E4). Time to first grade 2 or higher rifampin-related AE was also similar across arms (P=0.3610; Figure E1). The frequency and time to grade 2 or higher rifampin-related *hepatic* AEs and the frequency of SAEs were similar across arms (Tables 4-5). Results were similar when we excluded AEs that occurred during study halts in the experimental arms (Table E5).

The distribution of participants with at least one AE stratified by organ system is shown in Table E6. The most common AEs were hepatic (24.4%), gastrointestinal (14.4%), musculoskeletal (12.2%), and respiratory (11.7%). Nine SAEs occurred in eight participants during the entire study. Of these, six SAEs occurred in five participants during the safety period; four were rifampin-related (Table 5). A detailed description of all SAEs is provided in Table E7.

Discussion

The present study is the first clinical trial to show dose- and exposure-response of higher doses of rifampin under conditions of combination therapy. Higher rifampin doses and exposure resulted in an increase in the rate of sputum culture sterilization. These findings build on pharmacokinetic results from the present study and others showing that rifampin exposure increases at least proportionally with higher doses (25, 36), by clearly demonstrating that elimination rate is also related to rifampin $AUC_{0\text{-}6}$. These dose- and exposure-related efficacy findings are consistent with the results of the PanACEA MAMS-TB-01 trial (NCT01785186). This latter study found that rifampin doses of 20 mg/kg are unlikely to permit treatment shortening and that the optimal dose of rifampin is likely higher than 20 mg/kg (26). The statistically nonsignificant increase in culture negativity in the 20 mg/kg arm of the RIFATOX trial (ISRCTN55670677) also suggests that this dose may not be sufficient for treatment shortening (42).

The present study characterized the consistent impact of both rifampin dose and exposure on efficacy. Other analyses were not statistically significant: the analysis of 8-week culture conversion in LJ medium, the mITT analysis of dose and exposure as measured by $AUC_{0\text{-}6}$ and $AUC_{0\text{-}6}/MIC_{99.9}$ on the primary endpoint of change in log_{10}CFU/mL, and the PP analysis of exposure as measured by $AUC_{0\text{-}6}/MIC_{99.9}$. There are several potential explanations for these findings. First, the study was not powered for the 8-week binary, relatively insensitive, secondary endpoint. Second, the mITT efficacy estimates were affected by protocol-defined study halts. The use of standard rifampin dosing among all participants during study halts may have diminished the effect of the higher doses, biasing the effect estimate of dose towards the

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

null. This explanation is supported by the significant effect of dose detected in the PP analysis, which includes only the 132 (73.3%) participants who received their assigned rifampin dose for the full intensive phase. Study halts may have also biased the effect of exposure towards the null in the mITT population. Since rifampin exposure was not measured during study halts, the estimates of rifampin exposure in experimental arm participants who received standard rifampin dosing during study halts likely overestimate overall rifampin exposure. Given the effect of study halts on both dose- and exposure-response estimates in the mITT population, we feel that the PP population is less biased than the mITT population for the efficacy analysis, even though the PP population was the prespecified secondary analysis population.

Third, 1:1:1 randomization yielded median rifampin doses of 450, 900, and 975 mg in the 10, 15, and 20 mg/kg arms, respectively. Since median weights for all three arms fell in the 53-55 kg range, we expected that median rifampin doses would approach 600, 900, and 1200 mg, the assigned rifampin doses for the 55-70 kg weight band. However, more than half of study participants in the 10 mg/kg arm were in the two lowest rifampin weight bands, more than half in the 15 mg/kg arm were in the two highest weight bands, and the 20 mg/kg arm had the same number of participants in the two lowest and two highest weight bands. Consequently, the median difference in dose between the 15 mg/kg and 20 mg/kg arms was only 75 mg. These subtle differences in assigned rifampin doses (and consequent exposures) could have biased our results, reducing the apparent activity of the 20 mg/kg arm and the ability to distinguish a dose-response effect.

The effect of rifampin $AUC_{0-6}/MIC_{99.9}$ (the primary PK/PD endpoint) on sputum culture sterilization was of only borderline statistical significance. Prior preclinical studies have suggested that rifampin AUC/MIC is a strong predictor of concentration-dependent killing (22, 33). Clinical studies of patients receiving combination therapy have shown mixed results. Others have shown that rifampin AUC alone is an important predictor of clinical outcomes (32), and that its predictive ability may be bolstered by MIC information (34). However, a more recent study found no relationship between rifampin AUC/MIC and 2-month culture conversion (35). The distribution of susceptible rifampin MICs in our sample was higher than in the latter study (35), albeit within previously published ranges (34, 43). We did not find that rifampin MICs varied across levels of AUC to suggest that the effect of rifampin $AUC_{0-6}/MIC_{99.9}$ was attenuated by selection bias. Our findings suggest that rifampin $AUC_{0-6}/MIC_{99.9}$ was not as strong of a predictor of sputum sterilization as rifampin AUC_{0-6}, and that there may be a role for higher doses of rifampin in populations with susceptible rifampin MICs on the higher end of the distribution. In addition, the present analysis of rifampin $MIC_{99.9}$ does not capture the activity of rifampin metabolites which may contribute to treatment efficacy.

Despite our inclusion criterion of baseline sputum smear grade of 2+ or higher, we found a mean baseline mycobacterial load of 5.2 $\log_{10}$CFU/mL, approximately tenfold lower than in previous studies (8, 44). Low baseline loads have been reported in other settings, such as Hong Kong, Tanzania, and South Africa (25, 27, 45, 46). In the present study, low baseline loads may have contributed to the selection of a monophasic model of elimination by rifampin dose. This is distinct from the biphasic model selected to best represent the data in a study of fluoroquinolone-containing regimens (8). Our findings are consistent with those of a 14-day early bactericidal

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

activity study by the TB Alliance, which did not show a pronounced initial fall in the mycobacterial load among patients receiving standard therapy who had a mean baseline load of 5.399 $\log_{10}$CFU/mL (47), and by a recent observation that the 0-2 day early bactericidal activity of isoniazid-based treatments is strongly correlated with pretreatment mycobacterial load (46).

We found no difference in the secondary efficacy outcomes of 8-week culture conversion or in the frequency of treatment failure and disease recurrence across arms. The frequencies of treatment failure and recurrence we observed are comparable to those of standard therapy in other clinical trials (16, 19, 48). However, these relatively rare events occurred disproportionately in the 10 mg/kg arm, in which the median rifampin dose was 450 mg daily. We previously reported that 81% of the patients in the 20 mg/kg arm and only 33% of those of in the 10 mg/kg arm achieved a C_{max} of >8 mg/L, the low end of the targeted range (36). Others have reported that standard rifampin dosing through FDCs has resulted in suboptimal C_{max} <8 mg/L (35). Taken together, these findings suggest that standard doses of rifampin at 10 mg/kg may have compromised treatment efficacy and that the 20 mg/kg dose should be considered for dosing to maximize efficacy pending results of studies evaluating even higher doses. This has important implications for the use of FDCs, which are currently recommended over separate drug formulations in the treatment of drug-susceptible tuberculosis (49). Current guidelines recommend maximum rifampin doses of 600 mg daily (2), or 750 mg daily when using FDCs containing 150 mg of rifampin each (50). Under programmatic settings, supplementation with loose rifampin would be required to achieve higher doses.

We did not observe an increased risk of toxicity with rifampin doses up to 20 mg/kg delivered over 8 weeks. We found similar results when we excluded adverse events that occurred during study halts in the experimental arms. Our power calculation assumed a 10% frequency of grade 2 or higher rifampin-related AEs in the control arm. With 43.3% of control arm participants having at least one event, there was increased power over what was anticipated: we had more than 80% power to rule out a toxicity difference of 20% between the control arm and experimental arms. Our safety findings are consistent with recently published data from several studies. The maximum-dose tolerability study, HIGHRIF1 (NCT01392911), found no SAEs among participants who received rifampin doses up to 35 mg/kg over 14 days (25). The RIFATOX Phase II trial found that rifampin doses up to 20 mg/kg delivered over 16 weeks did not result in a significant increase in AEs (42). Finally, the more recent MAMS-TB-01 trial found that a rifampin dose of 35 mg/kg delivered as part of combination therapy over 12 weeks resulted in similar frequencies of grade 3 or higher AEs to standard therapy (26). Taken together, there is a growing body of evidence from controlled studies to suggest that concerns about the toxicity of rifampin may have taken on too much importance – relative to efficacy. In a future publication, we plan to pool data from this trial with those from another Phase II trial of high-dose rifampin conducted by PanACEA, HIGHRIF2 (NCT00760149) (27), with the goal of improving statistical power for efficacy and safety evaluations.

In summary, this is the first controlled trial to show dose- and exposure-response of higher doses of rifampin on sputum culture sterilization under conditions of combination therapy. Rifampin doses of up to 20 mg/kg/day were safe compared to the standard dose. These findings support

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

reconsideration of currently recommended standard dosing guidelines and the continued investigation of higher doses of rifampin, beyond 20 mg/kg, for potential treatment shortening.

Acknowledgements

We thank the patients and their families who made this study possible. We also thank the health care workers at the participating health centers in Lima Ciudad and Lima Este.

References

1. World Health Organization. *Global Tuberculosis Report 2017*. Geneva, Switzerland: World Health Organization; 2017. Report No. WHO/HTM/TB/2017.23.
2. World Health Organization. *Treatment of tuberculosis: guidelines - 4th edition*. Geneva: World Health Organization; 2010. Report No. WHO/HTM/TB/2009.420.
3. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946-1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 1999;3:S231–S279.
4. A Cooperative Tuberculosis Chemotherapy Study in Poland from the National Research Institute for Tuberculosis PSDoTaRD. A comparative study of daily followed by twice or once weekly regimens of ethambutol and rifampicin in retreatment of patients with pulmonary tuberculosis. The results at 1 year. A cooperative tuberculosis chemotherapy study in Poland. *Tubercle* 1975;56:1–26.
5. A Cooperative Tuberculosis Chemotherapy Study in Poland from the National Research Institute for Tuberculosis PSDoTaRD. A comparative study of daily followed by twice- or once-weekly regimens of ethambutol and rifampicin in the retreatment of patients with pulmonary tuberculosis: second report. *Tubercle* 1976;57:105–113.
6. Hong Kong Tuberculosis Treatment Services, Brompton Hospital, British Medical Research Council. A controlled trial of daily and intermittent rifampicin plus ethambutol in the retreatment of patients with pulmonary tuberculosis: results up to 30 months. *Tubercle* 1975;56:179–189.
7. van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, Gillespie SH, Boeree MJ. Why do we use 600 mg of rifampicin in tuberculosis treatment? *Clin Infect Dis* 2011;52:e194–199.
8. Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, Reddy C, Sturm AW, Sirgel FA, Allen J, Coleman DJ, Fourie B, Mitchison DA, Gatifloxacin for TB (OFLOTUB) study team. A Phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2008;12:128–138.
9. Nuermberger EL, Yoshimatsu T, Tyagi S, O'Brien RJ, Vernon AN, Chaisson RE, Bishai WR, Grosset JH. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *Am J Respir Crit Care Med* 2004;169:421–426.
10. Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. *Am J Respir Crit Care Med* 2004;170:1131–1134.
11. Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, Choudhri S, Daley CL, Munsiff SS, Zhao Z, Vernon A, Chaisson RE. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. *Am J Respir Crit Care Med* 2006;174:331–338.
12. Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, Bozeman L, Heilig CM, Bernardo J, Choudhri S, Grosset JH, Guy E, Guyadeen P, Leus MC, Maltas G, Menzies D, Nuermberger EL, Villarino M, Vernon A, Chaisson RE, Tuberculosis Trials Consortium. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. *Am J Respir Crit Care Med* 2009;180:273–280.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

13. Johnson JL, Hadad DJ, Dietze R, Maciel ELN, Sewali B, Gitta P, Okwera A, Mugerwa RD, Alcaneses MR, Quelapio MI, Tupasi TE, Horter L, Debanne SM, Eisenach KD, Boom WH. Shortening treatment in adults with noncavitary tuberculosis and 2-month culture conversion. *Am J Respir Crit Care Med* 2009;180:558–563.
14. Conde MB, Efron A, Loredo C, De Souza GRM, Graça NP, Cezar MC, Ram M, Chaudhary MA, Bishai WR, Kritski AL, Chaisson RE. Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. *Lancet* 2009;373:1183–1189.
15. Dorman SE, Goldberg S, Stout JE, Muzanyi G, Johnson JL, Weiner M, Bozeman L, Heilig CM, Feng P-J, Moro R, Narita M, Nahid P, Ray S, Bates E, Haile B, Nuermberger EL, Vernon A, Schluger NW, Tuberculosis Trials Consortium. Substitution of rifapentine for rifampin during intensive phase treatment of pulmonary tuberculosis: study 29 of the tuberculosis trials consortium. *J Infect Dis* 2012;206:1030–1040.
16. Jindani A, Harrison TS, Nunn AJ, Phillips PPJ, Churchyard GJ, Charalambous S, Hatherill M, Geldenhuys H, McIlleron HM, Zvada SP, Mungofa S, Shah NA, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk JH, Clouting HE, Coleman D, Bateson ALE, McHugh TD, Butcher PD, Mitchison DA, RIFAQUIN Trial Team. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. *N Engl J Med* 2014;371:1599–1608.
17. ClinicalTrials.gov. *TBTC Study 31: Rifapentine-containing Tuberculosis Treatment Shortening Regimens (S31/A5349)* [cited 2017 Dec 15]. Available from: https://clinicaltrials.gov/ct2/show/NCT02410772
18. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* 1985;66:219–225.
19. Jindani A, Nunn AJ, Enarson DA. Two 8-month regimens of chemotherapy for treatment of newly diagnosed pulmonary tuberculosis: international multicentre randomised trial. *Lancet* 2004;364:1244–1251.
20. Long MW, Snider DE, Farer LS. U.S. Public Health Service Cooperative trial of three rifampin-isoniazid regimens in treatment of pulmonary tuberculosis. *Am Rev Respir Dis* 1979;119:879–894.
21. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1980;121:939–949.
22. Jayaram R, Gaonkar S, Kaur P, Suresh BL, Mahesh BN, Jayashree R, Nandi V, Bharat S, Shandil RK, Kantharaj E, Balasubramanian V. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother* 2003;47:2118–2124.
23. Sirgel FA, Fourie PB, Donald PR, Padayatchi N, Rustomjee R, Levin J, Roscigno G, Norman J, McIlleron H, Mitchison DA. The early bactericidal activities of rifampin and rifapentine in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2005;172:128–135.
24. Poole G, Stradling P, Worlledge S. Potentially serious side effects of high-dose twice-weekly rifampicin. *Br Med J* 1971;3:343–347.
25. Boeree MJ, Diacon AH, Dawson R, Narunsky K, Bois du J, Venter A, Phillips PPJ, Gillespie SH, McHugh TD, Hoelscher M, Heinrich N, Rehal S, van Soolingen D, van Ingen J, Magis-Escurra C, Burger D, Plemper van Balen G, Aarnoutse RE, PanACEA Consortium. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. *Am J Respir Crit Care Med* 2015;191:1058–1065.

26. Boeree MJ, Heinrich N, Aarnoutse R, Diacon AH, Dawson R, Rehal S, Kibiki GS, Churchyard G, Sanne I, Ntinginya NE, Minja LT, Hunt RD, Charalambous S, Hanekom M, Semvua HH, Mpagama SG, Manyama C, Mtafya B, Reither K, Wallis RS, Venter A, Narunsky K, Mekota A, Henne S, Colbers A, van Balen GP, Gillespie SH, Phillips PPJ, Hoelscher M, *et al.* High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. *Lancet Infect Dis* 2017;17:39–49.
27. Aarnoutse RE, Kibiki GS, Reither K, Semvua HH, Haraka F, Mtabho CM, Mpagama SG, van den Boogaard J, Sumari-de Boer IM, Magis-Escurra C, Wattenberg M, Logger JGM, Brake te LHM, Hoelscher M, Gillespie SH, Colbers A, Phillips PPJ, Plemper van Balen G, Boeree MJ. Pharmacokinetics, tolerability, and bacteriological response of rifampin administered at 600, 900, and 1,200 milligrams daily in patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* 2017;61:e01054–17.
28. Requena-Méndez A, Davies G, Ardrey A, Jave O, López-Romero SL, Ward SA, Moore DAJ. Pharmacokinetics of rifampin in Peruvian tuberculosis patients with and without comorbid diabetes or HIV. *Antimicrob Agents Chemother* 2012;56:2357–2363.
29. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrob Agents Chemother* 2006;50:1170–1177.
30. Chigutsa E, Visser ME, Swart EC, Denti P, Pushpakom S, Egan D, Holford NHG, Smith PJ, Maartens G, Owen A, McIlleron H. The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin concentrations: dosing implications. *Antimicrob Agents Chemother* 2011;55:4122–4127.
31. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clin Infect Dis* 2012;55:169–177.
32. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis* 2013;208:1464–1473.
33. Gumbo T, Louie A, Deziel MR, Liu W, Parsons LM, Salfinger M, Drusano GL. Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. *Antimicrob Agents Chemother* 2007;51:3781–3788.
34. Gumbo T, Pasipanodya JG, Wash P, Burger A, McIlleron H. Redefining multidrug-resistant tuberculosis based on clinical response to combination therapy. *Antimicrob Agents Chemother* 2014;58:6111–6115.
35. Rockwood N, Pasipanodya JG, Denti P, Sirgel F, Lesosky M, Gumbo T, Meintjes G, McIlleron H, Wilkinson RJ. Concentration-dependent antagonism and culture conversion in pulmonary tuberculosis. *Clin Infect Dis* 2017;64:1350–1359.
36. Peloquin CA, Velásquez GE, Lecca L, Calderón RI, Coit J, Milstein MB, Osso E, Jimenez J, Tintaya K, Sánchez Garavito E, Vargas Vasquez D, Mitnick CD, Davies GR. Pharmacokinetic evidence from the HIRIF trial to support increased doses of rifampin for tuberculosis. *Antimicrob Agents Chemother* 2017;61:e00038–17.
37. Milstein MB, Velásquez GE, Coit JM, Jimenez J, Tintaya K, Osso EM, Davies GR, Mitnick CD. Tuberculosis clinical trials and safety halts: evaluating for bias in effect estimates [abstract]. Abstract OA-220-14. Presented at the 48th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Union). October 10–14, 2017, Guadalajara, Mexico. *Int J Tuberc Lung Dis* 2017;21(11 Suppl 2):S350–S351.
38. Milstein M, Coit J, Velásquez GE, Osso E, Vasquez DV, Garavito ES, Davies G, Mitnick C. Active screening for hepatotoxicity: identifying time periods for targeted monitoring in tuberculosis patients [abstract]. Abstract PD-722-13. Presented at the 48th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union). October 10–14, 2017, Guadalajara, Mexico. *Int J Tuberc Lung Dis* 2017;21(11 Suppl 2):S275–S276.
39. Velásquez GE, Brooks MB, Coit JM, Vargas Vasquez D, Sánchez Garavito E, Calderón RI, Jimenez J, Tintaya K, Peloquin CA, Osso E, Tierney DB, Seung KJ, Lecca L, Davies GR, Mitnick CD. A randomized controlled trial of high-dose rifampin for pulmonary tuberculosis [abstract]. Abstract 39LB. Presented at the Conference on Retroviruses and Opportunistic Infections, March 4–7, 2018, Boston, MA.
40. Milstein M, Lecca LW, Peloquin CA, Mitchison D, Seung K, Pagano M, Coleman D, Osso E, Coit J, Vargas Vasquez DE, Sanchez-Garavito E, Calderon RI, Contreras C, Davies G, Mitnick CD. Evaluation of high-dose rifampin in patients with new, smear-positive tuberculosis (HIRIF): study protocol for a randomized controlled trial. *BMC Infect Dis* 2016;16:453.
41. National Institute of Allergy and Infectious Diseases. *Division of Microbiology and Infectious Diseases Adult Toxicity Table, November 2007, Draft* [cited 2017 Dec 15]. Available from: https://www.niaid.nih.gov/sites/default/files/dmidadulttox.pdf
42. Jindani A, Borgulya G, de Patiño IW, Gonzales T, de Fernandes RA, Shrestha B, Atwine D, Bonnet M, Burgos M, Dubash F, Patel N, Checkley AM, Harrison TS, Mitchison D, International Consortium for Trials of Chemotherapeutic Agents in Tuberculosis, St George's University of London. A randomised Phase II trial to evaluate the toxicity of high-dose rifampicin to treat pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2016;20:832–838.
43. Schön T, Juréen P, Giske CG, Chryssanthou E, Sturegård E, Werngren J, Kahlmeter G, Hoffner SE, Angeby KA. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for Mycobacterium tuberculosis. *J Antimicrob Chemother* 2009;64:786–793.
44. Ruslami R, Nijland H, Aarnoutse R, Alisjahbana B, Soeroto AY, Ewalds S, van Crevel R. Evaluation of high- versus standard-dose rifampin in Indonesian patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* 2006;50:822–823.
45. Sirgel FA, Donald PR, Odhiambo J, Githui W, Umapathy KC, Paramasivan CN, Tam CM, Kam KM, Lam CW, Sole KM, Mitchison DA. A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. *J Antimicrob Chemother* 2000;45:859–870.
46. De Jager V, van der Merwe L, Venter A, Donald PR, Diacon AH. Time trends in sputum mycobacterial load and two-day bactericidal activity of isoniazid-containing antituberculosis therapies. *Antimicrob Agents Chemother* 2017;61:e02088–16.
47. Diacon AH, Dawson R, Groote-Bidlingmaier von F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Hutchings J, Burger DA, Schall R, Mendel CM. Bactericidal activity of pyrazinamide and clofazimine alone and in combinations with pretomanid and bedaquiline. *Am J Respir Crit Care Med* 2015;191:943–953.
48. Merle CS, Fielding K, Sow OB, Gninafon M, Lo MB, Mthiyane T, Odhiambo J, Amukoye E, Bah B, Kassa F, N'Diaye A, Rustomjee R, de Jong BC, Horton J, Perronne

C, Sismanidis C, Lapujade O, Olliaro PL, Lienhardt C, OFLOTUB/Gatifloxacin for Tuberculosis Project. A four-month gatifloxacin-containing regimen for treating tuberculosis. *N Engl J Med* 2014;371:1588–1598.

49. World Health Organization. *Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017 update*. Geneva, Switzerland: World Health Organization; 2017. Report No. WHO/HTM/TB/2017.05.
50. World Health Organization. *Operational guide for national tuberculosis control programmes on the introduction and use of fixed-dose combination drugs*. Geneva, Switzerland: World Health Organization; 2002. Report No. WHO/CDS/TB/2002.308.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Figure 1. Study flow for efficacy analyses.

*One patient had an indeterminate Hain result.

†Clinical or radiological signs suggestive of pericardial, pleural, or miliary TB.

‡Serum alanine aminotransferase > 2 times the upper limit of normal.

§Hemoglobin concentration < 7.0 g/dL or platelet count < 150,000/mm^3.

‖Screening was not completed in four patients due to the implementation of protocol-defined study halts, and in one patient due to an inoperative Hain machine.

CFU = colony forming units; ET = early termination; INH = isoniazid; LTFU = lost to follow up; PK = pharmacokinetics; RIF = rifampin; TB = tuberculosis.

Figure 2. Predictions of bacillary elimination for the three treatment arms derived from the partial likelihood model showing observed data (dark grey) and model predictions (light grey) above and below the limit of detection of colony counting, respectively.

Table 1. Participant characteristics by treatment arm.*

Characteristic	Rifampin Dose 10 mg/kg *N* = 60	Rifampin Dose 15 mg/kg *N* = 60	Rifampin Dose 20 mg/kg *N* = 60	Total *N* = 180
Age (years), median (IQR)	24 (21-37)	25 (20-35)	27 (22-37)	25 (21-37)
Female	21 (35.0)	19 (31.7)	26 (43.3)	66 (36.7)
Weight (kg), median (IQR)†	53.5 (50.6-59.6)	55.4 (49.2-60.5)	54.9 (50.3-61.7)	54.1 (50.5-60.8)
BMI (kg/m^2), median (IQR)†	21.2 (19.2-23.3)	21.2 (19.5-23.9)	21.4 (19.9-24.2)	21.2 (19.6-23.7)
Cavitary disease (*n* = 178)	23 (39.0)	22 (37.3)	21 (35.0)	66 (37.1)
HIV positive	2 (3.3)	2 (3.3)	1 (1.7)	5 (2.8)
Diabetes (*n* = 179)	0 (0)	0 (0)	1 (1.7)	1 (0.6)
Smear grade at baseline				
++	32 (53.3)	24 (40.0)	31 (51.7)	87 (48.3)
+++	28 (46.7)	36 (60.0)	29 (48.3)	93 (51.7)
Baseline log_{10}CFU/mL, median (IQR) (*n* = 175)	5.0 (4.4-5.8)	5.0 (4.5-6.2)	5.1 (4.6-5.8)	5.0 (4.5-5.9)
Baseline TTP (days), median (IQR)	4.3 (1.6-5.0)	4.0 (0.1-5.2)	4.4 (0.1-5.0)	4.3 (0.1-5.0)
$MIC_{99.9}$, median (IQR)	0.2 (0.1-0.2)	0.2 (0.1-0.3)	0.2 (0.1-0.2)	0.2 (0.1-0.2)
$AUC_{0\text{-}6}/MIC_{99.9}$, median (IQR) (*n* = 168)	115.7 (59.1-197.6)	202.0 (144.1-460.3)	284.4 (175.3-399.2)	193.8 (112.0-344.4)
Rifampin dose (mg), median (IQR)	450 (450-600)	900 (600-900)	975 (750-1200)	750 (600-900)

Definition of abbreviations: AUC, area under the plasma concentration-time curve; BMI, body mass index; CFU, colony forming units; HIV, human immunodeficiency virus; MIC, minimum inhibitory concentration; TTP, time-to-positivity in BACTEC MGIT 960.

* Values shown are number (%) unless otherwise specified.

† Values for weight and BMI shown were those obtained at the randomization visit.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table 2. Decrease in viable CFU counts of *M. tuberculosis* by rifampin dose or exposure to rifampin.

Dose/Exposure Variable	*N*	$\Delta\log_{10}$ CFU/mL/day	95% CI*	*P* value
5 mg/kg increase in rifampin dose				
mITT population	174	-0.011	-0.025 – 0.002	0.230
PP population	132	-0.022	-0.046 – -0.002	0.022
1 log increase in rifampin $AUC_{0\text{-}6}$				
mITT population	163	-0.001	-0.003 – 0.001	0.750
PP population	126	-0.017	-0.029 – -0.007	0.011
1 log increase in rifampin $AUC_{0\text{-}6}/MIC_{99.9}$				
mITT population	163	-0.002	-0.005 – 0.002	0.330
PP population	126	-0.010	-0.021 – 0.000	0.053

Definition of abbreviations: AUC, area under the plasma concentration-time curve; CFU, colony forming units; CI, confidence interval; mITT, modified intention-to-treat; MIC, minimum inhibitory concentration; PP; per-protocol.

* 95% CI were obtained using the sandwich estimator.

Table 3. Secondary efficacy outcomes.*

Outcome	Rifampin Dose 10 mg/kg *N* = 60	15 mg/kg *N* = 60	20 mg/kg *N* = 60	Total *N* = 180
Culture conversion in Löwenstein-Jensen medium at 8 weeks				
Converted	46 (76.7)	44 (73.3)	45 (75.0)	135 (75.0)
Did not convert	3 (5.0)	5 (8.3)	7 (11.7)	15 (8.3)
Contaminated week 8 cultures	4 (6.7)	1 (1.7)	0 (0)	5 (2.8)
Week 8 cultures not available	7 (11.7)	10 (16.7)	8 (13.3)	25 (13.9)
Early discontinuation	6 (10.0)	9 (15.0)	8 (13.3)	23 (12.8)
Lost to follow up	1 (1.7)	1 (1.7)	0 (0)	2 (1.1)
Treatment outcome at 12 months				
Recurrence-free cure	44 (73.3)	46 (76.7)	46 (76.7)	136 (75.6)
Treatment failure	3 (5.0)	1 (1.7)	1 (1.7)	5 (2.8)
Recurrence after cure	3 (5.0)	1 (1.7)	2 (3.3)	6 (3.3)
Relapse[†]	2 (3.3)	0 (0)	0 (0)	2 (1.1)
Reinfection[‡]	0 (0)	1 (1.7)	0 (0)	1 (0.6)
Recurrence[§]	1 (1.7)	0 (0)	2 (3.3)	3 (1.7)
Death	0 (0)	0 (0)	0 (0)	0 (0)
Outcome not evaluable	10 (16.7)	12 (20.0)	11 (18.3)	33 (18.3)
Early discontinuation	8 (13.3)	11 (18.3)	10 (16.7)	29 (16.1)
Lost to follow up	2 (3.3)	1 (1.7)	1 (1.7)	4 (2.2)

*Values shown are No. (%).

[†]2 patients had relapse diagnosed by identical pre-treatment and post-treatment *M. tuberculosis* isolates, with 24/24 matching MIRU-VNTR loci.

[‡]1 patient had reinfection diagnosed by nonidentical pre-treatment and post-treatment *M. tuberculosis* isolates, with 9/20 matching MIRU-VNTR loci.

[§]Recurrent tuberculosis was diagnosed without positive culture (and without MIRU-VNTR) in 3 patients: 2 were diagnosed by a positive sputum smear, and 1 was diagnosed clinically.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table 4. Participants experiencing grade 2 or higher rifampin-related adverse events and hepatic adverse events during the safety period, by treatment arm.*

	Rifampin Dose			
Variable	10 mg/kg *N* = 60	15 mg/kg *N* = 60	20 mg/kg *N* = 60	*P* value
≥ 1 grade 2+ RIF-related AE	26 (43.3)	31 (51.7)	23 (38.3)	0.7092†
Time to first grade 2+ RIF-related AE (days), median (IQR)	29.5 (14-39)	26 (9-42)	25 (13-37)	0.3610‡
≥ 1 grade 2+ hepatic RIF-related AE	16 (26.7)	14 (23.3)	14 (23.3)	0.6645†
Time to first grade 2+ RIF-related hepatic AE (days), median (IQR)	31.5 (14-40.5)	36.5 (26-57)	42.5 (26-63)	0.8357‡

Definition of abbreviations: AE, adverse event; RIF, rifampin.

* Values shown are No. (%) unless otherwise specified. The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

† One-sided Cochran-Armitage test for trend with 5% significance.

‡ Global log-rank test.

Table 5. Participants experiencing serious adverse events by treatment arm.*

	Rifampin Dose			
Variable	10 mg/kg $N = 60$	15 mg/kg $N = 60$	20 mg/kg $N = 60$	P value†
≥ 1 SAE during entire study	2 (3.3)	1 (1.7)	5 (8.3)	0.0919
≥ 1 SAE during safety period‡	1 (1.7)	1 (1.7)	3 (5.0)	0.1333
≥ 1 RIF-related SAE during safety period‡	1 (1.7)	1 (1.7)	2 (3.3)	0.2679

Definition of abbreviations: RIF, rifampin; SAE, serious adverse event.

* Values shown are No. (%).

† One-sided Cochran-Armitage test for trend with 5% significance.

‡ The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

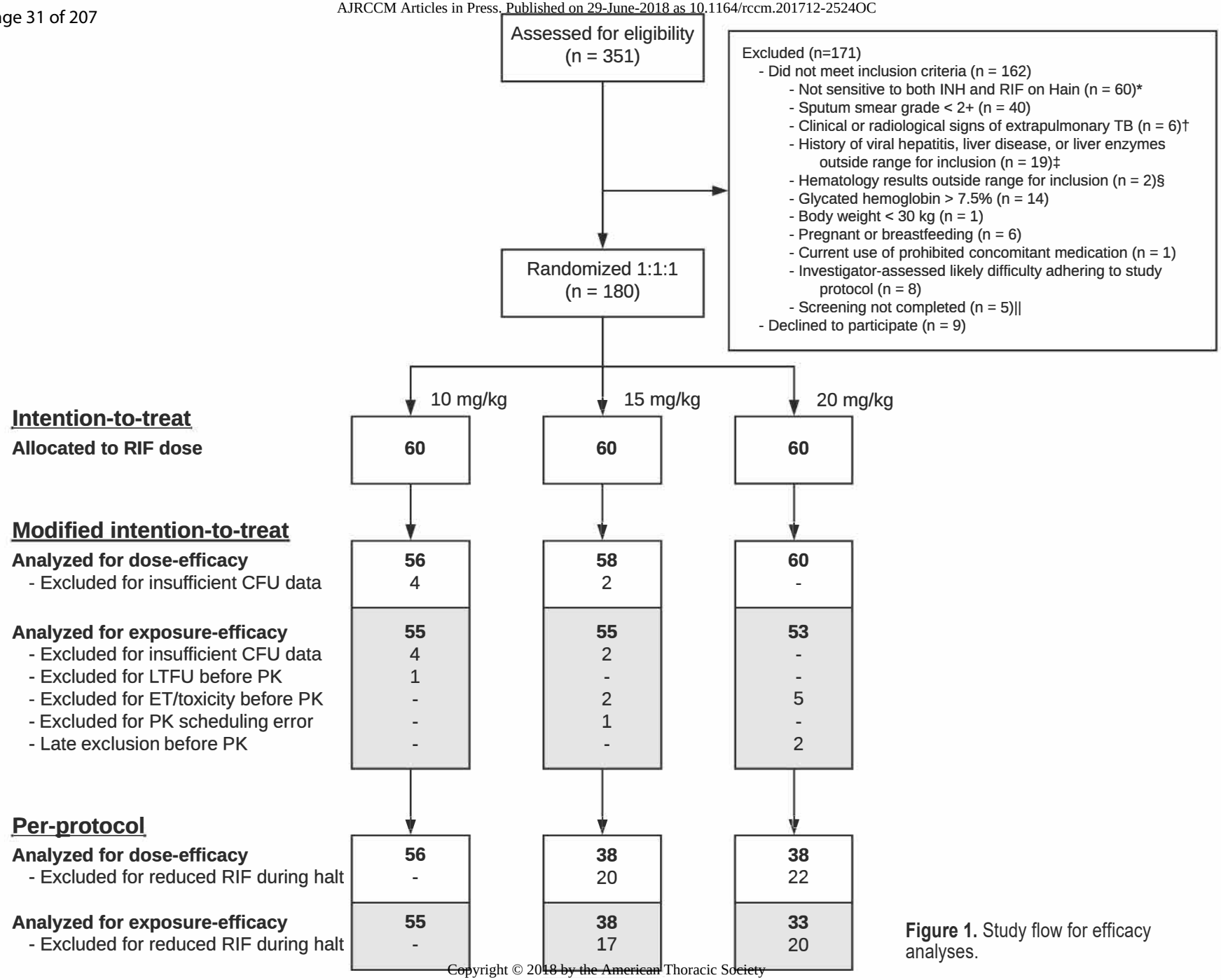


Figure 1. Study flow for efficacy analyses.

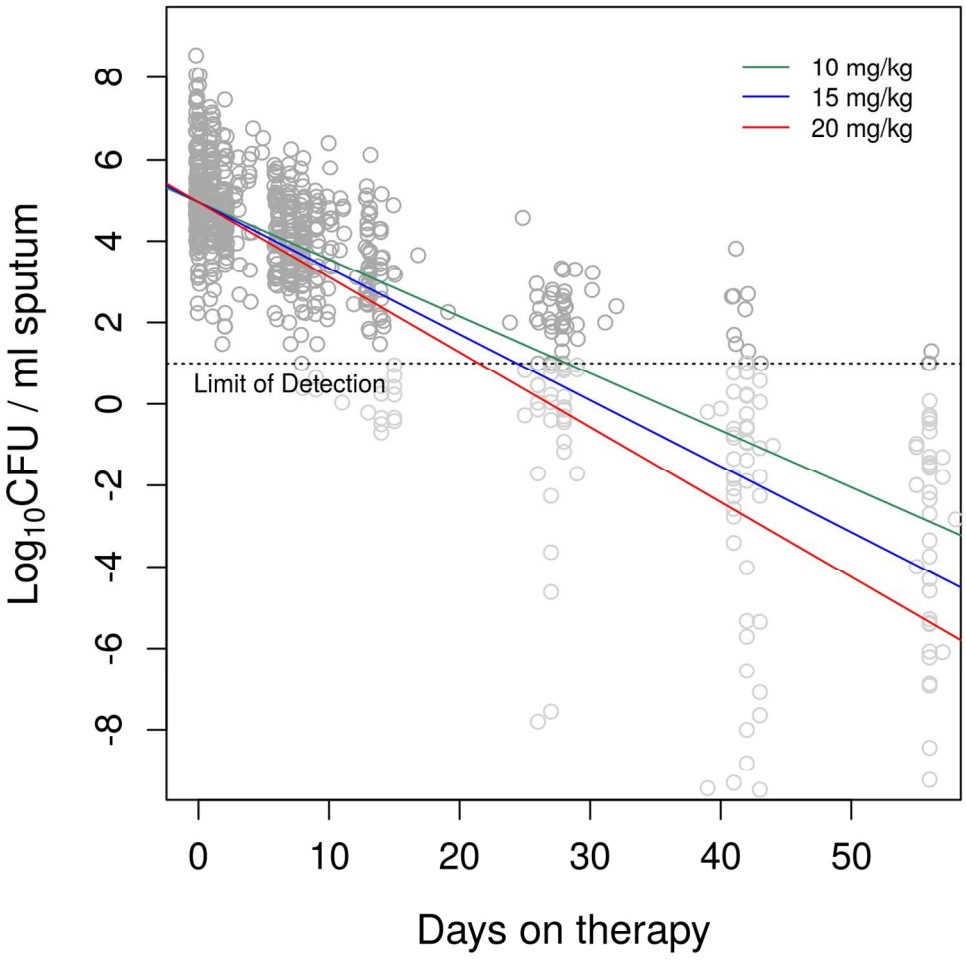


Figure 2. Predictions of bacillary elimination for the three treatment arms derived from the partial likelihood model showing observed data (dark grey) and model predictions (light grey) above and below the limit of detection of colony counting, respectively.

134x134mm (300 x 300 DPI)

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Online Data Supplement

Methods

Study setting and design

The study protocol for "Evaluation of high-dose rifampin in patients with new, smear-positive tuberculosis" (HIRIF) has been published previously (E1). We conducted a multi-site, triple-blind, randomized, controlled Phase II clinical trial and here we report trial findings in accordance with CONSORT guidelines (E2). A CONSORT checklist and the full study protocol are provided with the online supplement.

We enrolled patients with newly diagnosed, previously untreated, smear-positive (grade 2+ or higher) pulmonary tuberculosis with susceptibility to isoniazid and rifampin detected by Hain GenoType MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) between September 12, 2013 and February 18, 2015 from two health districts of Lima, Peru (Lima Ciudad and Lima Este). Participants were identified and referred from 43 health centers to Hospital Nacional Hipólito Unanue (HNHU) or Hospital Sergio Bernales (HNSEB) for eligibility screening.

Exclusion criteria

Exclusion criteria were weight <30 kg; age <18 years or >60 years; rifampin resistance; previous tuberculosis treatment for ≥1 month; extrapulmonary manifestations (central nervous system, miliary, pericardial, or pleural tuberculosis); significant hemoptysis; study drug intolerance, hypersensitivity, or contraindication; pregnancy or breastfeeding; alanine aminotransferase >2 times the upper limit of normal (ULN); total bilirubin >2.5 times ULN; creatinine >2 times ULN or clearance <60 mL/min; hemoglobin <7.0 g/dL; platelets <150,000/mm^3; white blood cell

count <4500 cells/μL; hepatitis B surface antigen or hepatitis C antibody seropositive; glycated hemoglobin >7.5%; or Karnofsky score <50. Additional criteria for study withdrawal included any patient without culture confirmation of *M. tuberculosis*, or whose isolate was found to be resistant to any of the study drugs after study treatment was initiated. If a participant's isolate tested resistant to ethambutol or pyrazinamide, in the absence of resistance to isoniazid or rifampin, confirmation of the resistant result was required prior to termination from the study.

Tuberculosis treatment

Participants received directly observed ambulatory treatment administered by treatment supporters and were randomized 1:1:1 in blocks (of varying size) to receive 10, 15, and 20 mg/kg of rifampin delivered orally, 7 days per week for 8 weeks. Participants also received standard oral doses of isoniazid 5 mg/kg/day, pyrazinamide 25 mg/kg/day, and ethambutol 20 mg/kg/day for 8 weeks; a continuation phase of isoniazid 10 mg/kg/day and rifampin 10 mg/kg thrice weekly for 18 weeks; and pyridoxine 50mg thrice weekly throughout treatment.

Pharmacokinetic methods

We performed pharmacokinetic (PK) measurements on a single day after steady state rifampin exposure was achieved, between 14 and 56 days after randomization. We randomized participants in a 2:1 ratio to sparse versus intensive PK measurements. Patients were asked to fast for four hours prior to measurements and for two hours after drug administration. Sparse PK measurements involved blood draws at three time points at 0, 2, and 6 hours after the last dose. Intensive PK measurements involved blood draws at four additional time points up to 14+ hours after the last dose. Blood samples were stored at -80°C and assayed at the University of Florida.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Rifampin concentrations were measured with a validated high-performance liquid chromatography assay and triple-quadrupole mass spectrometry. Phoenix v6.2 (Certara LP, Princeton, NJ) was used for noncompartmental analysis to derive rifampin PK parameters (E3).

Culture methods

We conducted study mycobacteriology at the Socios En Salud laboratory (Lima, Peru), which performs external quality assurance annually through proficiency panels from the Peruvian National Reference Laboratory and the College of American Pathologists (Northfield, Illinois). We obtained duplicate pretreatment samples through early-morning spot sputum and overnight sputum collections. Participants were followed at scheduled study visits once weekly for the first eight weeks of the intensive phase of treatment and monthly thereafter until 12 months after randomization. Participants provided sputum samples at baseline and at five time points during the first two months of treatment, depending on random assignment to one of two collection groups (at days 1-2, 3-7, 8-10, 14-16, and 40-44 for collection group 1; at days 1-2, 3-7, 9-11, 26-30, and 54-66 for collection group 2). We obtained additional early-morning sputum samples for smear microscopy (at week 8) and culture in Löwenstein-Jensen (LJ) medium (at weeks 2, 4, 8, and monthly thereafter until month 12). We also used the BACTEC™ MGIT™ 960 (MGIT) method (BD Diagnostics, Sparks, MD) on neat samples to estimate time-to-positivity (TTP) at baseline. We obtained four measures of rifampin minimum inhibitory concentration ([MIC], two replicates each from one pretreatment spot sputum and one pretreatment overnight sputum sample) and used the arithmetic mean of the pretreatment overnight collection MICs for the pharmacodynamic analyses. We used 7H11 Middlebrook medium to quantify *M. tuberculosis* colony forming units per mL of sputum (CFU/mL) in sputum culture to estimate sterilization.

We assumed that contaminated Middlebrook cultures were missing at random. The limit of detection of the colony counting method was 10 CFU/mL. For participants with recurrent disease, we sent the baseline strain and the post-recurrence strain to GenoScreen (Lille, France) for 24-locus mycobacterial interspersed repetitive units-variable number tandem repeat (MIRU-VNTR) sequencing using standard methods (E4).

Safety methods

Study staff, including treatment supporters, triaged solicited and unsolicited adverse events (AEs) at every participant contact, which occurred at scheduled study visits, daily treatment support visits during the intensive phase, and thrice weekly treatment support visits during the continuation phase of treatment. Staff recorded the use of concomitant medications, pregnancy, and breastfeeding at scheduled study visits. Safety assessments included complete blood count with differential, urinalysis, serum alanine aminotransferase, serum aspartate aminotransferase, serum alkaline phosphatase, total bilirubin, albumin, creatinine, and clinician-observed and unsolicited adverse events reported at or between study visits. Clinical investigators graded clinical and laboratory AEs according to the Adult Toxicity Table by the Division of Microbiology and Infectious Diseases of the National Institute of Allergy and Infectious Diseases (E5), and determined the relatedness of AEs to study drugs.

Efficacy and safety outcome classification

The primary efficacy endpoint was change in *M. tuberculosis* $\log_{10}$CFU/mL in sputum at 8 weeks on 7H11 Middlebrook medium. The secondary efficacy endpoint reported here is culture negativity at 8 weeks on LJ medium. We classified patients who had two contaminated samples

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

as not having culture conversion. The primary safety endpoint was the frequency of grade 2 or higher rifampin-related adverse events during the 8-week intensive phase of treatment and up to 4 weeks later. Secondary efficacy endpoints included time to grade 2 or higher rifampin-related adverse events, and the frequency and timing of grade 2 or higher rifampin-related hepatic adverse events.

Power and sample size

As stated in the protocol, a sample size of 180 participants, randomized in a 1:1:1 allocation across three arms and with 17% expected loss to follow-up, afforded 90% power to detect a 14 mcg/mL*hr expected difference in AUC between the lowest and highest rifampin dose, 80% power to detect a 0.025 $\log_{10}$CFU/mL expected difference in colony counts, and 68% power to rule out a two-fold difference in frequency of grade 2 or higher AEs between the control (assuming frequency of 10%) and intervention arms.

Interim analyses

An early safety evaluation by the data safety and monitoring board (DSMB) was performed after the first 60 patients completed 4 weeks of study treatment. Safety data were reviewed again during protocol-defined study halts. In addition, the DSMB reviewed trial progress after 120 participants completed 4 weeks of study treatment. An interim, blinded PK analysis was performed by G.R.D. when PK data were available for half of the patients to check: [1] the PK sampling, handling, and laboratory processing procedures, and [2] that the assumptions of the PK sampling scheme (especially for sparse sampling) were correct. This interim analysis was not

performed to make comparisons between groups. No interim efficacy analysis was planned or performed.

Protocol changes

After trial commencement, the protocol was changed to specify that resistance to ethambutol and pyrazinamide in the absence of resistance to isoniazid and rifampin would be confirmed prior to termination from the study. There were no changes to trial outcomes after the trial commenced.

Model building strategy for primary efficacy analysis

Fitted monophasic (A) and biphasic (B) structural models for colony counting data used a doubly-exponentiated parameterization to enforce positivity of the rate constants and improve numerical stability:

$$y_t = \log_{10}\left(e^{\theta_1} \times e^{-t \times e^{\theta_2}}\right) \quad \text{(A)}$$

$$y_t = \log_{10}\left(e^{\theta_1} \times e^{-t \times e^{\theta_2}} + e^{\theta_3} \times e^{-t \times e^{\theta_4}}\right) \quad \text{(B)}$$

Additive, combined additive and proportional and variance function error models were tested as were different random effect structures. Nested structural and covariate models were compared using the likelihood ratio test based on the change in the value of the objective function and the function *pchisq* in R. Standard errors were derived using the sandwich estimator implemented in the NONMEM covariance step and confirmed by nonparametric bootstrapping. Regression

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

diagnostics included examination of correlation and covariance matrices and condition numbers, plots of population and individual predictions against the data, and visual predictive checks.

Ethics statement

The study protocol and informed consent documents were reviewed and approved by the Partners HealthCare Human Research Committee (approval number 2011P001577), the Liverpool School of Tropical Medicine Research Ethics Committee and the University of Liverpool Research Support Office (approval number RETH000725), Institutional Ethics Committees at HNHU and University of San Martin de Porres (under ceding agreement with the study hospital, HNSEB), the Peruvian National Institute of Health (approval number 081–12) and the Division of Microbiology and Infectious Diseases, National Institutes of Allergy and Infectious Diseases, U.S. National Institutes of Health (protocol number 11–0050). The study received exemption from the University of Florida Institutional Review Board (exemption number IRB201200199).

References

E1. Milstein M, Lecca LW, Peloquin CA, Mitchison D, Seung K, Pagano M, Coleman D, Osso E, Coit J, Vargas Vasquez DE, Sanchez-Garavito E, Calderon RI, Contreras C, Davies G, Mitnick CD. Evaluation of high-dose rifampin in patients with new, smear-positive tuberculosis (HIRIF): study protocol for a randomized controlled trial. *BMC Infect Dis* 2016;16:453.

E2. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010;340:c869.

E3. Peloquin CA, Velásquez GE, Lecca L, Calderón RI, Coit J, Milstein MB, Osso E, Jimenez J, Tintaya K, Sánchez Garavito E, Vargas Vasquez D, Mitnick CD, Davies GR. Pharmacokinetic evidence from the HIRIF trial to support increased doses of rifampin for tuberculosis. *Antimicrob Agents Chemother* 2017;61:e00038–17.

E4. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C, van

Soolingen D. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. *J Clin Microbiol* 2006;44:4498–4510.

E5. National Institute of Allergy and Infectious Diseases. *Division of Microbiology and Infectious Diseases Adult Toxicity Table, November 2007, Draft* [cited 2017 Dec 15]. Available from: https://www.niaid.nih.gov/sites/default/files/dmidadulttox.pdf

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table E1. Dosing of rifampin and companion drugs in milligrams, by study arm and weight band.

				Study arm		
	Companion drugs			Control	Experimental 1	Experimental 2
Weight band (kg)	INH	PZA	EMB	RIF 10 mg/kg	RIF 15 mg/kg	RIF 20 mg/kg
30-37	150	800	550	300	450	600
38-54	225	1200	825	450	600	750
55-70	300	1600	1100	600	900	1200
>70	375	2000	1375	750	1200	1500

Definition of abbreviations: EMB = ethambutol; INH = isoniazid; PZA = pyrazinamide; RIF = rifampin.

Table E2. Description of study halts.*

Halt No.	Halt Duration	Reason for Halt
1	1.9 weeks	2 or more SAEs of the same type which were related to rifampin
2	2.3 weeks	6 or more grade 3 or higher AEs which were related to rifampin, of which 3 or more were due to elevated transaminases and had clinical symptoms of hepatotoxicity
3	5.4 weeks	6 or more grade 3 or higher AEs which were related to rifampin, of which 3 or more were due to elevated transaminases and had clinical symptoms of hepatotoxicity

Definition of abbreviations: AEs = adverse events; SAEs = serious adverse events.

* Study halts occurred respectively from December 27, 2013 to January 8, 2014; April 23 to May 8, 2014; and August 13 to September 19, 2014.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table E3. Description of all treatment failure and recurrence outcomes during study.

Patient No.	Treatment Arm	Rifampin Dose	Baseline Smear	log_{10}CFU/mL	Outcome*	Months†
1	10 mg/kg	450 mg	+++	4.66	Treatment failure	4.7
2	10 mg/kg	450 mg	+++	6.34	Treatment failure	4.7
3	10 mg/kg	450 mg	+++	8.05	Treatment failure	5.7
4	10 mg/kg	450 mg	+++	4.31	Relapse (pan-susceptible)	11.8
5	10 mg/kg	450 mg	+++	4.99	Recurrence‡	11.7
6	10 mg/kg	600 mg	+++	7.22	Relapse (pan-susceptible)	10.8
7	15 mg/kg	900 mg	+++	6.29	Treatment failure	5.9
8	15 mg/kg	900 mg	++	5.47	Reinfection	8.8
9	20 mg/kg	750 mg	+++	5.09	Recurrence‡	12.1
10	20 mg/kg	1200 mg	++	4.93	Treatment failure	5.1
11	20 mg/kg	1500 mg	+++	7.17	Recurrence‡	8.7

*Drug-susceptibility testing was not routinely performed for treatment failure at the study laboratory.

†Months from date of randomization to date of outcome.

‡Recurrent tuberculosis was diagnosed without positive culture (and without MIRU-VNTR) in 3 patients: One patient had a positive sputum smear at week 14; one patient had a positive sputum smear at week 15; and one patient had neither positive smear nor culture but was diagnosed clinically and restarted treatment outside the catchment area of the study.

Table E4. Participants experiencing grade 2 or higher rifampin-related adverse events and hepatic adverse events during the safety period, by treatment arm.*

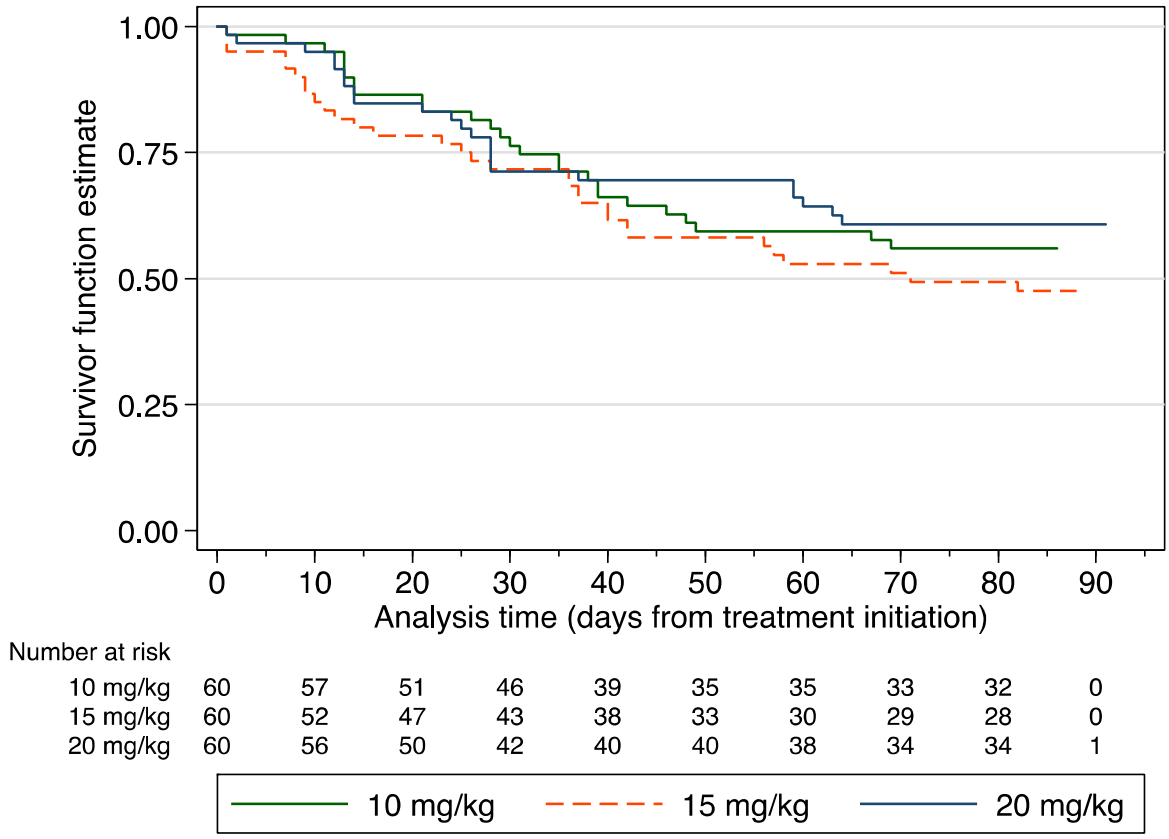
	Rifampin Dose			
Variable	10 mg/kg *N* = 60	15 mg/kg *N* = 60	20 mg/kg *N* = 60	Total *N* = 180
No. of grade 2+ RIF-related AEs				
None	34 (56.7)	29 (48.3)	37 (61.7)	100 (55.6)
One	21 (35.0)	22 (36.7)	16 (26.7)	59 (32.8)
Two	4 (6.7)	7 (11.7)	5 (8.3)	16 (8.9)
Three or more	1 (1.7)	2 (3.3)	2 (3.3)	5 (2.8)
No. of grade 2+ hepatic RIF-related AEs				
None	44 (73.3)	46 (76.7)	46 (76.7)	136 (75.6)
One	15 (25.0)	14 (23.3)	14 (23.3)	43 (23.9)
Two	1 (1.7)	0 (0)	0 (0)	1 (0.6)

Definition of abbreviations: AE = adverse event; RIF = rifampin.

*Values shown are No. (%) unless otherwise specified. The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Figure E1. Time to first grade 2 or higher rifampin-related adverse event during the safety period, by treatment arm.*



*The number of events per arm were 26 (10 mg/kg), 31 (15 mg/kg), and 23 (20 mg/kg). The global log-rank test comparing the three arms showed a $P = 0.3610$. The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

Table E5. Sensitivity analysis of participants experiencing grade 2 or higher rifampin-related adverse events, hepatic adverse events, and serious adverse events during the safety period, excluding events occurring during study halts in the 15 and 20 mg/kg arms.*

	Rifampin Dose			
Variable	10 mg/kg *N* = 60	15 mg/kg *N* = 60	20 mg/kg *N* = 60	*P* value
≥ 1 grade 2+ RIF-related AE	26 (43.3)	30 (50.0)	22 (36.7)	0.7694†
Time to first grade 2+ RIF-related AE (days), median (IQR)	29.5 (14-39)	25.5 (9-42)	25.5 (13-37)	0.3642‡
≥ 1 grade 2+ hepatic RIF-related AE	16 (26.7)	12 (20.0)	14 (23.3)	0.6670†
Time to first grade 2+ RIF-related hepatic AE (days), median (IQR)	31.5 (14-40.5)	36.5 (20-57.5)	42.5 (26-63)	0.6379‡
≥ 1 RIF-related SAE	1 (1.7)	1 (1.7)	2 (3.3)	0.2679†

Definition of abbreviations: AE = adverse event; RIF = rifampin.

*Values shown are No. (%) unless otherwise specified. The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

†One-sided Cochran-Armitage test for trend with 5% significance.

‡Global log-rank test.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table E6. Participants experiencing at least one grade 2 or higher adverse event during the safety period, by organ system and treatment arm.*

	Rifampin Dose			
Organ System	10 mg/kg *N* = 60	15 mg/kg *N* = 60	20 mg/kg *N* = 60	Total *N* = 180
Dermatological	7 (11.7)	2 (3.3)	10 (16.7)	19 (10.6)
Gastrointestinal	6 (10.0)	15 (25.0)	5 (8.3)	26 (14.4)
Hematological	3 (5.0)	4 (6.7)	5 (8.3)	12 (6.7)
Hepatic	16 (26.7)	14 (23.3)	14 (23.3)	44 (24.4)
Immunological	0 (0)	0 (0)	1 (1.7)	1 (0.6)
Musculoskeletal	7 (11.7)	7 (11.7)	8 (13.3)	22 (12.2)
Neurological	3 (5.0)	3 (5.0)	2 (3.3)	8 (4.4)
OB-GYN	0 (0)	1 (1.7)	0 (0)	1 (0.6)
Psychiatric	1 (1.7)	0 (0)	0 (0)	1 (0.6)
Respiratory	5 (8.3)	8 (13.3)	8 (13.3)	21 (11.7)
Urological	1 (1.7)	2 (3.3)	2 (3.3)	5 (2.8)
Other NOS	7 (11.7)	7 (11.7)	6 (10.0)	20 (11.1)

Definition of abbreviations: OB-GYN = obstetric and gynecological; NOS = not otherwise specified.

*Values shown are No. (%). The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

Table E7. Description of all serious adverse events during study.

SAE No.	Patient No.	Treatment Arm	SAE in Safety Period*	RIF Related	Organ System	SAE Category	Description
1	1	10 mg/kg	No	No	Respiratory	Required hospitalization or emergency care	Bronchial hyperreactivity, hospitalization
2	2	10 mg/kg	Yes	Yes	Hepatic	Other important medical event	Drug-induced hepatotoxicity
3	3	15 mg/kg	Yes	Yes	Hepatic	Other important medical event	Drug induced hepatotoxicity
4	4	20 mg/kg	Yes	Yes	Hepatic	Other important medical event	Drug-induced hepatotoxicity
5	4	20 mg/kg	Yes	No	Respiratory	Required hospitalization or emergency care	Left pneumothorax, hospitalization
6	5	20 mg/kg	No	No	OB-GYN	Other important medical event	Spontaneous abortion
7	6	20 mg/kg	No	No	Neurological	Persistent or significant incapacity or substantial disruption of the ability conduct normal life functions	Hearing loss
8	7	20 mg/kg	Yes	No	Respiratory	Required hospitalization or emergency care	Hemoptysis, hospitalization
9	8	20 mg/kg	Yes	Yes	Hepatic	Other important medical event	Drug-induced hepatotoxicity

Definition of abbreviations: OB-GYN = obstetric and gynecological; RIF = rifampin; SAE = serious adverse event.
*The safety period was defined as 12 weeks after randomization or 4 weeks after the last experimental rifampin dose, whichever occurred later.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Title page
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1-2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5-6; E2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	E4
Participants	4a	Eligibility criteria for participants	5; E2
	4b	Settings and locations where the data were collected	5; E2
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-6; E2-E3; Table E1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7; E3-E4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	E4
Sample size	7a	How sample size was determined	E4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	6; E4; Table E2
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5; E2
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5; E2
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5; E2
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5-6; E2
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	5-6; E2-E3; Table E1
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7; E4-E5
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7; E3-E5
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8; Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	8; Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5; E2; Table E2
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8; Figure 1; Tables 1-5, E2, E4-E6; Figure E1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9-10; Tables 2-5, E3-E7; Figure E1
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	9-10; Tables 3-5, E3-E7
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	9-10; Tables E3, E5-E7
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10; Tables 3-5, E2-E7; Figure E1
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11-16
Other information			
Registration	23	Registration number and name of trial registry	5

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Protocol	24	Where the full trial protocol can be accessed, if available	E2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Title page, 5

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

TITLE:

TRIPLE BLIND, RANDOMIZED, DOSE-RANGING TRIAL OF HIGH-DOSE RIFAMPIN IN PATIENTS WITH NEW, SMEAR-POSITIVE TB (HIGH DOSE RIFAMPIN OR HIRIF STUDY)

DMID Protocol Number: ***11-0050***

DMID Funding Mechanism: *1U01AI091429-01A1*

Investigators: *Carole Mitnick (co-PI), Geraint Davies (co-PI)*

Pharmaceutical Support Provided by: *sanofi aventis*

IND Sponsor: *Carole Mitnick, Harvard Medical School*

DMID Clinical Project Manager and Program Officer: *Robin Mason*

Draft or Version Number: *7.0*

Day Month Year
September 25, 2014

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

STATEMENT OF COMPLIANCE

This trial will be conducted with human subjects oversight from the following Institutional Review Boards (IRBs):

Harvard Medical School

Ethics Committee of the Hospital Nacional Hipólito Unanue and the University of San Martin de Porres-Clínica Cada Mujer, Peru

The study will be carried out in accordance with the following guidelines and regulations:

- Compliance with the International Conference on Harmonisation (ICH) E6: Good Clinical Practice (GCP): Consolidated Guideline and the applicable regulatory requirements.
- Compliance with the United States Code of Federal Regulations (CFR) applicable to clinical studies (Title 45 CFR Part 46 and Title 21 CFR including Parts 50 and 56 concerning informed consent and Institutional Review Board (IRB) regulations, 21 CFR 312).
- NIH Clinical Terms of Award
- Regulations of Clinical Trials in Peru. Supreme Decree No. 017-2006-SA; amendment to the Regulation of Clinical Trials in Peru. Supreme Decree No. 006-2007-SA.
- Relevant regulations in the UK

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Co-Principal Investigator

Signed: Date: _____

_____

Name: Carole Mitnick, Sc.D.
Title: Associate Professor
Department of Global Health and Social Medicine
Harvard Medical School

Co-Principal Investigator

Signed: Date: _____

_____

Name: Geraint Davies, B.M, Ph.D., MRCP, DTM&H
Title: Senior Lecturer in Infectious Disease
Honorary Consultant Physician
School of Clinical Sciences
University of Liverpool

Peru Investigator

Signed: Date: _____

_____

Name: Leonid Lecca, M.D
Title: Research Associate
Socios En Salud Sucursal in
Lima, Perú

Peru Clinical Site Investigators

Signed: Date: _____

_____

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Name: Dante Elmo Vargas Vásquez, M.D
Title: Pulmonologist
Hospital Nacional Hipólito Unanue in
Lima, Perú

Signed: Date: _____

_____

Name: Epifanio Sánchez Garavito, MD
Title: Pulmonologist
Hospital Nacional Sergio E. Bernales – Lima, Perú

St. George's, UK Investigator

Signed: Date: _____

_____

Name: Denis Mitchison, CMG FRCP FRCPath
Title: Professor of Medical Microbiology
Department of Cellular and Molecular Medicine
St. George's University of London

U. Florida Investigator

Signed: Date: _____

_____

Name: Charles Peloquin, Pharm. D.
Title: Director of Infectious Disease Pharmacokinetics Laboratory
College of Pharmacy and Emerging Pathogens Institute
University of Florida

Table of Contents

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

TABLE OF FIGURES

LIST OF ABBREVIATIONS

7H11 medium	Agar medium for growth of Mycobacterium tuberculosis
ADL	Activities of Daily Living
AE	Adverse Event/Adverse Experience
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATS	American Thoracic Society
AUC	Area under the plasma concentration-time curve
BACTEC 7H12B	Becton-Dickinson Agar Medium type, Blood Culture System
ßHCG	Beta human chorionic gonadotropin
CBC	Complete Blood Count
CD4	Cluster of Differentiation 4, a human T cell marker"
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CFU	Colony Forming Units
C_{max}	Observed maximum plasma concentration of drug
CRF	Case Report Form
CYP	Cytochrome P450 enzyme
DCC	Data Coordinating Center at Harvard University
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DOT	Directly Observed Therapy
DSE	Direct Smear Examination
DST	Drug Susceptibility Test

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DTP	Days to Positivity
DTT	Dithiothreitol
EBA	Early bactericidal activity
EDTA	Ethylenediaminetetraacetic Acid or Edetic Acid
E, EMB	Ethambutol
EMA	European Medicines Agency
FDA	Food and Drug Administration
FDC	Fixed Dose Combination
GC/MS	Gas chromatography/mass spectrometry
GCP	Good Clinical Practice
HAIN test	Line probe assay to test for resistance to isoniazid and rifampin
HbA1c	Glycocylated hemoglobin
HBVsAg	Hepatitis B virus surface antigen
HCVAb	Hepatitis C virus antibody
HEZ	Isoniazid, Ethambutol and Pyrazinamide
HIRIF	Study name acronym
HIV	Human Immunodeficiency Virus
H, INH	Isoniazid
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
J774A.1	Mouse Macrophage Cell Line
LFT	Liver Function Test
LOD	Limit of Detection
LTBI	Latent Tuberculosis Infection

MGIT	Mycobacterial Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit-Variable Number
MOP	Manual of Procedures
N	Number (refers to subjects in table)
NALC	N-acetyl-L-cysteine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NLME	Non-Linear Mixed Effects
NONMEM VI	Non-Linear Mixed Effects Modeling Software
PA-824	Investigational anti-TB compound (nitroimidazole)
PAE	Post-Antibiotic Effect
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PCR	Polymerase Chain Reaction
PXR	Pregnane X Receptor
PZA	Pyrazinamide
R, RIF	Rifampin/Rifampicin
RPNT	Rifapentine
SAE	Serious Adverse Event/Serious Adverse Experience
SES	Socios En Salud
SM	Streptomycin
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SQ-109	Investigational anti-TB compound (Ethambutol derivative)
SSCC	Serial sputum colony counting
T_{max}	Time of maximum plasma concentration
TB	Tuberculosis

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

US	United States
USPHS	United States Public Health Service
WHO	World Health Organization
Z	Pyrazinamide

PROTOCOL SUMMARY

Title: Triple Blind, Randomized, Dose-Ranging Trial of High-Dose Rifampin in Patients with New, Smear-Positive TB (High Dose Rifampin or HIRIF Study)

Phase: II

Population: New, smear-positive, pulmonary TB patients aged between 18-60 years

Number of Sites: 2

Site Names: Socios En Salud Sucursal Perú, Executing Institution; Research Center at Hospital Nacional Hipólito Unanue and Center for Research on Lung Disease at Hospital Nacional Sergio E. Bernales, Peru Study Sites

Location of Sites: Lima, Peru

Study Duration: 3 years

Participant Duration: 12 months

Description of Agent or Intervention: High-dose rifampin (15 and 20 mg/kg/day)

Objectives:

Across three, daily, oral doses of RIF (10, 15 and 20 mg/kg/day):

Primary:

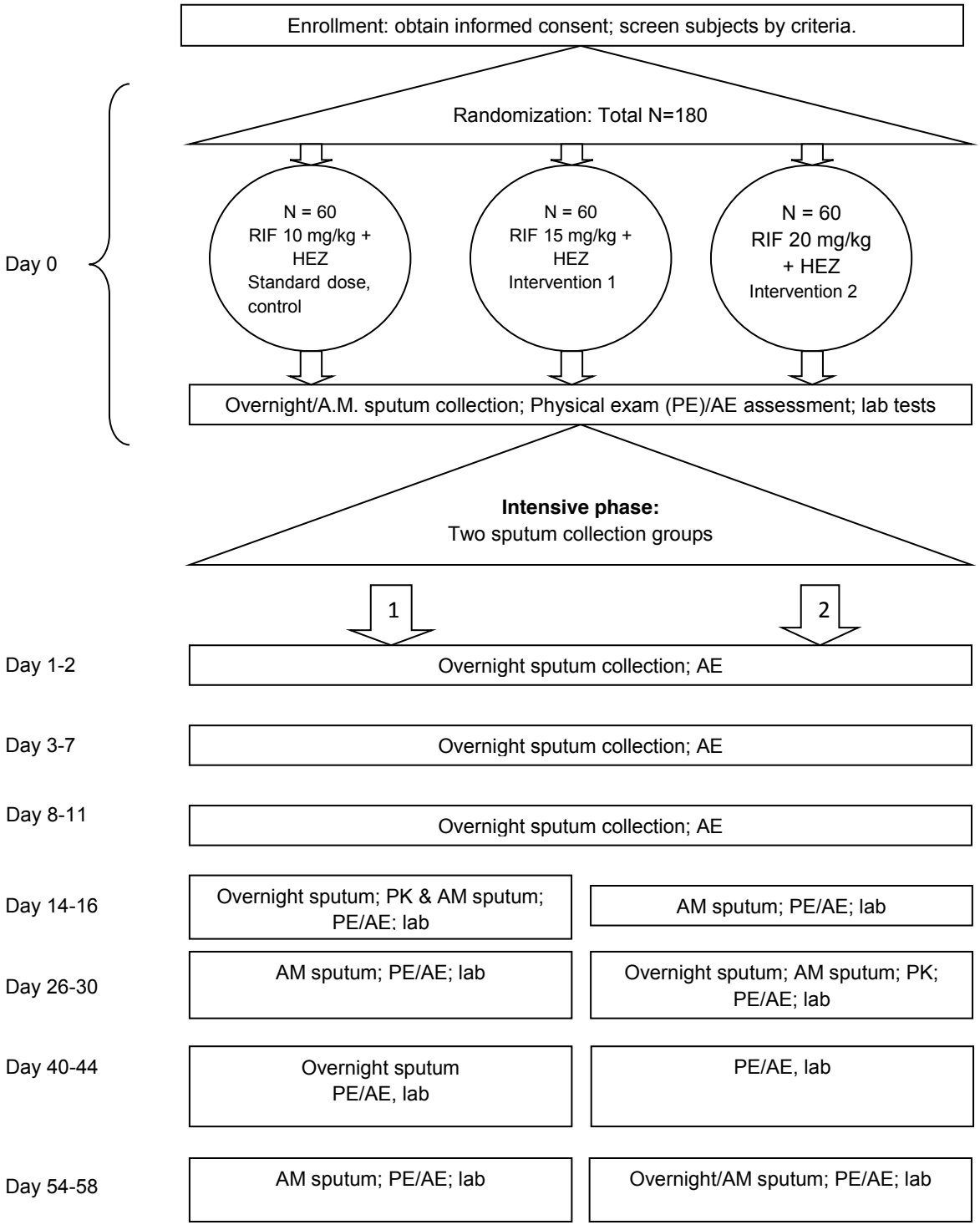
1. To assess the difference in steady state pharmacokinetic exposure of RIF and 25-desacetyl-rifampin.
2. To assess the difference in sputum culture sterilization during the initial 8 weeks.
3. To compare the incidence of adverse events related to study drug or regimen during the initial 8 weeks of daily four-drug treatment, and up to four weeks later.

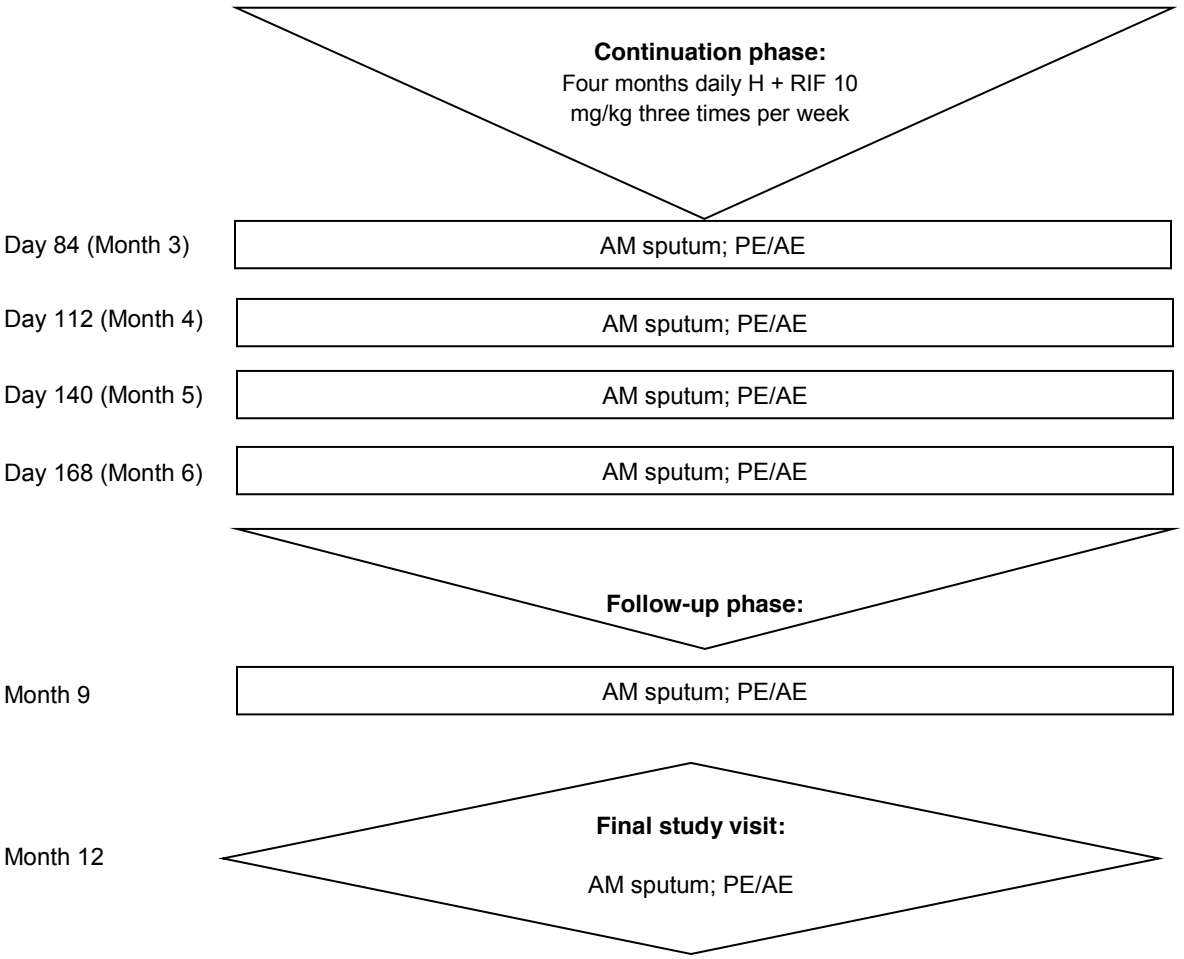
Secondary:

1. To estimate the relationship between pharmacokinetic parameters and measures of sputum culture sterilization.
2. To compare 4 methods for estimating sterilizing activity: 1) decline in log colony-forming units (CFUs), 2) speed of culture conversion in solid & liquid media, 3) increased time to detection in liquid medium, and 4) proportion of patients with positive cultures at 8 weeks.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

SCHEMATIC OF STUDY DESIGN





"HEZ": Standard doses of standard companion drugs, isoniazid, pyrazinamide, ethambutol; "PE": Physical examination; "AE": Assessment for adverse events; "Overnight sputum": overnight pooled sputum collection as inpatient; "AM sputum": early morning collection (unpooled) sample; lab: blood chemistry and hematology and urine testing (see schedule of events, Figure 2).

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

1.0 KEY ROLES

Individuals:

Co-Principal Investigator:
Carole Mitnick, Sc.D.
Associate Professor
Department of Global Health and Social Medicine
Harvard Medical School
641 Huntington Ave., 3rd Floor
Boston, MA 02115
P.: (617) 432-6018
F.: (617) 432-2565
Email: Carole_Mitnick@hms.harvard.edu

Co-Principal Investigator:
Geraint Davies, M.B., Ph.D., MRCP, DTM&H
Senior Lecturer in Infectious Diseases
Honorary Consultant Physician
School of Clinical Sciences
University of Liverpool
Liverpool L69 3BX
United Kingdom
P.: +0151-706-3036
F.: +0151-706-5944
Email: Gerrydavies@doctors.org.uk

Sponsor: National Institutes of Allergy and Infectious Diseases,
National Institutes of Health

Clinical Project Manager and Program Officer:
Robin Mason
NIAID/DMID/RDB
6610 Rockledge Drive, #3224
Bethesda, MD 20892
P.: (301) 451-3746
F.: (301) 496-8030
Email: rmason@niaid.nih.gov

Medical Monitor:
Mohamed Elsafy, M.D, MSc
DMID/NIAID/OCRA
6610 Rockledge Drive, #4409
Bethesda, MD 20892
P.: (301) 451-3026
F.: (301) 480-0728
Email: mo.elsafy@nih.gov

Medical Officer:
Alan C. Ou, MD, MPH
Commander, US Public Health Service
NIAID/DMID/RDB/TOMDS
6610 Rockledge Drive, #3204
Bethesda, MD 20892
P: (301) 451-3346
F: (301) 496-8030
Email: alan.ou@nih.gov

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Institutions:

Executing Institution:

Socios En Salud Sucursal Perú,
Avenida Merino. Reyna 575, Carabayllo,
Lima 6, Perú
Leonid Lecca, M.D.
P.: +511-651-6512
F.: +511-481-4478
Email: llecca_ses@pih.org

Peru Study Sites:

Centro de Investigación del Hospital Nacional Hipólito Unanue
Av. César Vallejo 1390, El Agustino Lima-Perú
Dante Elmo Vargas Vásquez, M.D.
P.: +511-6527516 or -6527517
F.: +511-627-9429
Email: dantevargasv_4@hotmail.com

Centro de Investigación del Hospital Nacional Sergio E. Bernales
Av. Tupac Amaru Km 14.5, Comas Lima-Perú
Epifanio Sánchez Garavito, M.D.
P.: +511-6377182 or -6377183
F.: +511-5580490
Email: epis53@yahoo.es

Other Study Sites:

St. George's University of London
Cranmer Terrace, London, SW180RE, UK
Denis A. Mitchison, C.M.G., B.M., FRCP Path
P.: +44-208-725-5704
F.: +44-208-672-0234
Email: dmitchis@sgul.ac.uk

University of Florida
Infectious Disease Pharmacokinetics Laboratory
College of Pharmacy and Emerging Pathogens Institute
1600 SW Archer Rd., Rm P4-33
Gainesville, FL 32610-0486
Charles Peloquin, Pharm. D

P.: (352) 273-6266
F.: (352) 273-6804
Email: peloquin@cop.ufl.edu

2.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Limitations of current tuberculosis treatment

More than 9 million cases of TB occur each year; despite the availability of effective treatment, 2 million of these result in death.[1] The standard of care for TB in most high-burden regions is a six-month, four-drug regimen, well supported by clinical trials completed more than three decades ago.[2] In particular, the successful introduction of rifampin (RIF) and pyrazinamide (PZA) allowed the reduction of treatment duration from 18 to 6 months with a risk of relapse frequency of <5%. Further reductions in treatment duration, however, resulted in increasing rates of relapse[3] suggesting that, with standard doses of RIF (10mg/kg) and PZA (25mg/kg), the duration of treatment could not be less than 6 months.

These "short-course" regimens offered vast improvements over previously used treatments in both delivery of care and clinical outcomes. In 1993, the 6-month regimen became the cornerstone of the World Health Organization (WHO) response to the TB emergency. In 2007, 5.5 million TB cases were treated with short-course chemotherapy. The current duration of treatment requires sustained support from health workers and committed adherence to the regimen from patients. Although on average 85% of patients treated in 2006 experienced successful outcomes, many patients cannot complete or are not cured by the six-month regimen.[1] Subsequently, these patients may require more prolonged re-treatment with less chance of success and the risk of acquisition of drug resistance. Additional information on limitations of current treatment and work underway to address them is provided in section 17.1.

This study will evaluate several potential surrogate endpoints for failure/relapse: culture conversion in solid medium at 2 months, time to culture conversion in solid medium, change in days to positivity in MGIT, and change in log colony forming units (CFU) in solid, selective medium. The first two semi-quantitative measures will be performed on early morning sputum samples. The latter two will be conducted on pooled sputum samples collected overnight. The pooled sputum increases the heterogeneity of the sample, which is appropriate for these fully quantitative measures.

2.2 Rationale

This trial will examine the concept that an increased dose of RIF could shorten the course of standard therapy for TB, and improve treatment outcomes, without increased toxicity. Specifically, we hypothesize that steady state pharmacokinetic parameters will demonstrate greater exposure of RIF among participants who receive 15 and 20 mg/kg/day of RIF compared to participants who receive the standard dose of 10 mg/kg/day. In addition, the impact of dose and concentration on the incidence of adverse events will be assessed. The shortening concept will be examined by evaluating surrogate measures of sterilizing activity—defined theoretically by Mitchison as, "the ability to kill *all* or virtually all of the bacilli in the lesions as rapidly as possible"[4]—of the higher doses of RIF against standard doses. Sterilizing activity is considered the primary determinant of length of TB treatment (additional information on sterilizing activity is provided in section 17.2). If sterilizing activity is enhanced in the first two months, this will be interpreted as proof of concept that tuberculosis treatment can be shortened. Several different methods will be employed to evaluate sterilizing activity. This study will compare and examine the relationships between these measures of sterilizing activity and pharmacokinetic parameters as well as long-term treatment outcomes.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

2.2.1 High-dose rifampin for treatment shortening

It is clear that individual anti-tuberculosis drugs make distinct contributions to the sterilizing activity of combination regimens. Of the four current first-line agents, RIF has the most potent and durable sterilizing activity and is the only drug for which available evidence suggests that an increase in dose size might be obtained without a substantial increase in adverse effects.[5-8] There is a clear relationship between the duration of continuous administration of rifampin and treatment success under current dosing recommendations.[9, 10] However, selection of the current daily dose of 600 mg appears to have been based on incomplete dose-finding studies and considerations of cost. Several recent reviews summarize the evidence that higher doses of RIF could successfully shorten TB treatment.[11-15]

In vitro, animal and human data all support the concept that treatment shortening with higher RIF dose may be expected. This evidence is summarized below; further detail is provided in section 17.3.

2.2.1.1 *Evidence from in vitro experiments*

Rifamycins exhibit concentration-dependent killing *in vitro* analogous to that seen with aminoglycosides and fluoroquinolones.[16, 17] Jayaram et al. showed that *M. tuberculosis* killing by RIF was influenced both by concentration up to the highest concentration tested (256 mcg/mL) and time up to 9 days (see *Figure 5* and *Figure 6* in section 17.3.1).[5] This recent data is consistent with an earlier study by Dickinson et al (which inferred *M. tuberculosis* killing from decreased uptake of uridine-^{14}C) that showed increased bactericidal activity with RIF concentrations from 0.625 to 4 mcg/mL.[18]

2.2.1.2 *Evidence from animal experiments*

Dramatically improved sterilizing activity and survival were achieved in the mouse and the guinea pig with increased RIF doses.[19-22] Grumbach and colleagues reported sterilization of all mice treated with 25 mg/kg RIF and 5 mg/kg INH for 6 months, while a regimen containing only 5 mg/kg RIF had been found to be inadequate. Kradolfer then identified 5.6 mg/kg as the threshold for a "chemotherapeutic effect" and found that increasing RIF doses increased mouse survival more than did increasing INH doses. Studies by Verbist revealed dose-related killing of *M. tuberculosis* in mice given RIF 5 to 40 mg/kg: a 2-log increase in killing occurred when the 5 mg/kg dose was doubled (see *Figure 5* in section 17.3.1).

2.2.1.3 *Evidence from human pharmacokinetics*

RIF is normally absorbed completely when taken orally, but food delays absorption. After 1.5 to 2 hours, a 600 mg dose yields a peak blood level of 8-24 mcg/mL.[23] The half-life of RIF varies from 2 to 5 hours, and it is shortened by approximately 20-40% after the first week of daily treatment.

RIF exposure is known to be dose-related and at least dose-proportional in the ranges so far examined in clinical studies.[24] Though the extent to which metabolism of RIF is saturated at current doses has not been fully characterized, the available data suggest that RIF induction of hepatic microsomal enzymes may approach a maximum effect at doses as low as 150-300 mg.[25-28] Though higher doses of RIF modestly shorten the time to full induction, the absolute amount of induction is not changed (see Table 10 in section 17.3.3).[29] An EBA study revealed a near quadrupling of AUC/MIC when RIF dose was increased from 300 to 600 mg.[7] In a recent observational study by Ruslami et al., AUC increased by a factor of 1.65 when RIF dose was increased from 450 to 600 mg.[30] Decroix et al also reported that increasing the RIF dose from 600 to 900 mg resulted in a near doubling of serum concentrations during the entire two months when concentrations were monitored.[31] Acocella observed significant saturation of RIF first-pass metabolism as doses were increased beyond the standard 10 mg/kg dose. By doubling the RIF dose to 20

mg/kg, the maximum serum concentration (C_{max}) was tripled.[32] Thus, significant increases in exposure are to be expected from even modest increases in dose.

2.2.1.4 *Evidence from efficacy studies*

A series of clinical studies evaluated treatment response and toxicity over a range of increased doses of RIF and provided some evidence that sterilizing activity may also be accelerated. The observed increase in pharmacokinetic exposure with dose has been linked to improved response using markers of efficacy in humans. In studies of early bactericidal activity, a linear increase in the activity of RIF has been reproducibly demonstrated up to a dose of 1200 mg[33] and the other rifamycins (rifapentine, rifabutin) show similar behavior. This contrasts with the dose-response curve repeatedly observed for isoniazid which exhibits a clear plateau of activity above 300 mg daily.[34] In sum, efficacy studies reveal that RIF doses higher than 450 mg, 600 mg or 10 mg/kg are associated with increased the frequency of culture conversion at both months 1 and 2. These data, therefore, support the concept that doses greater than 600 mg may increase culture conversion during the first two months, consistent with the results obtained in animal models. (For more information, see Table 10 in section 17.3.3)

2.2.2 Substudies

The rationale for three bacteriologic substudies is presented here. Their methods and execution are described in Section 4.4.

2.2.2.1 *LipTox Red Staining*

The identification of biomarkers that may replace or extend the usefulness of standard or quantitative bacteriology for the purpose of measuring the sterilizing activity of anti-tuberculosis regimens is a current research priority. An ability to more clearly identify the effect of sterilizing drugs on persister subpopulations of organisms in clinical specimens would be highly advantageous in the early clinical development phase. Such a biomarker may be better able to predict stable cure than current culture-based bacteriological methods.

It is now well-documented that *Mycobacterium tuberculosis*, like most bacteria, exhibits a variable phenotype depending on environmental conditions. In particular a number of different conditions including hypoxia, nutrient starvation and drug pressure induce a reasonably conserved transcriptional response coordinated via a two-component system by the *dosR* regulon. This is accompanied by diminished or absent growth, antibiotic tolerance and a profound switch in energy metabolism in favor of beta-oxidation of fatty acids. Morphologically this phenotype can be identified *in vitro* and *in vivo* by intracellular accumulation of lipid bodies containing tri-acyl glycerol.

A simple method for visualizing these lipid bodies has been developed using the neutral lipid stain LipTox Red and characterized both in vivo and in vitro. More extensive evaluation in clinical studies is needed to establish whether this method could be used to follow changes in persister sub-populations of bacilli during the course of therapy, how these changes compare with quantitative bacteriology using solid and liquid media and whether they can predict long-term outcome. The causal interpretation of such evaluation studies would be facilitated by dose-ranging designs of acknowledged sterilizing anti-tuberculosis drugs that could in principle demonstrate a dose-response relationship on this biomarker.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

2.2.2.2 *Comparison of culture methods*

EBA studies that used decontamination prior to plating[35, 36] have reported ¼-½ log less 0-2 day activity than studies in which decontamination was not used[6, 37-40] (see **Table 1** below). This may be because decontamination selectively kills the bacilli that would otherwise be killed in the early phase of treatment. Any killing due to decontamination would affect estimates of early bactericidal activity, possibly reducing the apparent effect of a new drug. To date, however, the effect of decontamination has not been observed on the bacilli that persist in the late phase; it is the bactericidal activity during the late phase that is the main criterion of whether treatment can be shortened.

SSCC is a labor- and space-intensive method requiring substantial incubator space and time of laboratory workers to count colonies on the plates. Change in time to detection using the MGIT procedure, especially when performed with the machine that provides automated readouts, represents a much less labor intensive approach to quantifying (with greater sensitivity than with standard semi-quantitative culture) changes in bacterial load.

The proposed substudy will examine the dynamic process of elimination of bacteria using three bacteriologic endpoints: conventional SSCC, SSCC with decontamination, and change in time to detection with MGIT.

Table 1 ***Range of 0-2 day EBA by decontamination approach***

Ref	Decontamination or digestion reported	Baseline CFU	0-2 EBA
Jindani et al, Nairobi[6]	No	Not reported	0.72
Sirgel et al, S. Africa[37]	No	6.32	0.50
Chan et al, Hong Kong[38]	No	Not reported	0.43
Hafner et al, US[35]	NALC	6.69	0.27
Kennedy et al, Tanzania[36]	NAOH	6.38	0.25 (estimated from 7-day)
Johnson et al, Brazil[39]	No	6.74	0.57
Dietze et al, Brazil[40]	No	6.52	0.67

2.2.2.3 *Mixed strain infections*

Recent studies indicate that *M. tuberculosis* infections are more complex than had previously been appreciated: an individual can be infected with more than one strain and each strain can evolve during the course of infection.[41, 42] Little is currently known about the prevalence of complex infections, the pathogen- and host-factors related to complex infections, or the effect of complex infections on the treatment outcomes of individuals or on the performance of strategies to control disease spread in

communities. The substudy proposed here will assess the pretreatment prevalence of complex infections and the impact of complex infection on early treatment response.

Although DNA extraction methods from culture-negative sputum samples have been compared,[43] methods of specimen collection that will yield multiple strains, if multiple strains are present, have yet to be refined. Pooling of sputum samples collected from a single patient over several hours—the technique employed in this study at 6 time points for each participant—may optimize the ability to extract DNA from multiple strains. Comparing the results of strain genotyping from a pretreatment sample and from subsequent pooled samples (after more than 1 week of treatment) will permit assessment of selective killing of a subset of strains early in treatment. These results will be linked to the development of mathematical models designed to assess the effect of complex *M. tuberculosis* infection on the projected performance of new strategies for TB control.

2.3 Potential Risks and Benefits of HIRIF

Subjects in all arms will receive at least the minimum curative therapy for TB; no decrease in treatment efficacy, over the current standard, is expected. The four-drug, curative regimen that will be administered to all subjects is the standard of care for drug-susceptible TB and has been used for >30 years. In the Peru study site, approximately 90% of patients receiving this regimen are consistently cured (Bonilla, C., *personal communication*, 2005). Increased doses of RIF may improve the probability of cure. There are potential risks of the study related to the safety of using higher doses of RIF. There are other risks of the study related to confidentiality and study procedures. They are all discussed below.

2.3.1 Potential Risks

2.3.1.1 *Toxicity data from animals*

Though dose-dependent toxicity of RIF has been observed in animals, RIF doses associated with such increases in toxicity were in the range of 100-200 mg/kg or, at least 6.67 times higher than the 15 or 20 mg/kg doses proposed for this trial. Acute toxicity of RIF has been examined in mice, rats, guinea pigs and rabbits. Median lethal dose varied dramatically depending on animal and route of administration, but was generally at least 900 mg/kg with oral administration in all the animals. Intravenous administration led to mortality in half the animals at doses ranging from 260 mg/kg (in mice) to 639 mg/kg in guinea pigs.[44-46] Additional animal toxicity data are provided in section 17.4.1.

2.3.1.2 *Toxicity data from humans: studies of tuberculosis*

Clinical safety data support the concept that serious toxicities of rifampicin are uncommon and not dose-related. At a daily dose of 10 mg/kg (maximum 600 mg), RIF is well tolerated with <10% of patients experiencing significant adverse reactions. Gastrointestinal adverse effects are the most common (1-10 percent), and include epigastric distress, anorexia, nausea, vomiting, cramps, and diarrhea. Anecdotal evidence suggests that GI side-effects are not dose related. In six patients not responding to standard doses of antituberculous therapy, RIF doses were raised from 600 mg to 900 mg, and in one patient, to 1500 mg. Although three patients were alcoholics and one was HIV-infected, all responded to therapy and no adverse effects or poor outcomes were experienced.[47] Kimerling and colleagues reported a similar experience with patients in whom RIF doses were raised in response to low serum concentrations of RIF on standard therapy. At least one patient ultimately received RIF at 1800 mg/day with no reported adverse events.[48] Red-orange discoloration of tears, sweat, saliva, feces, and urine is also common and may lead to staining of contact lenses.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Serious toxicities attributed to RIF, particularly hepatotoxicity and flu-like syndrome, are not believed to be dose-related. RIF hepatotoxicity appears to be idiosyncratic.[49] Known risk factors include advanced age, alcohol consumption, diabetes, and concomitant hepatotoxic agents. Although there are some inconclusive reports of increased incidence of hepatotoxicity with RIF and INH used in combination,[49-55] available data do not support an increase in hepatotoxicity with higher doses of RIF. Some studies have found increased risk of liver-function abnormality among TB patients with sub-clinical Hepatitis B or C (positive serology, no clinical symptoms, and transaminases within normal range) compared to those with negative Hepatitis B or C serology. These studies were, however, inconclusive about risk or severity of hepatotoxicity. And, they demonstrated that these patients can safely be treated with RIF-containing regimens.[56-58] Moreover, there are no reports of concentration- or exposure-dependent hepatotoxicity among this important subpopulation.

The flu-like syndrome is believed to be related to dosing interval and not dose size even at doses up to 1800 mg. It is hypothesized to be immunologic in nature: an extended interval between the doses may induce hypersensitivity while daily dosing permits tolerance.[59] The flu-like syndrome has been observed predominantly in situations where elevated doses are highly intermittent (once or twice weekly), either by design, or because of patient non-adherence to treatment.[16, 60-64] Clinical studies of increased doses of RIF which also reported toxicity are summarized in section 17.3.3.

Other severe adverse events, including thrombocytopenia, hemolytic anemia, and acute renal failure, also may occur, and these require permanent discontinuation of RIF.[16, 49, 65] No evidence of increased frequency of these events with higher daily doses of RIF has been reported.[8] Additional information is provided in section 17.4.2.

2.3.1.3 *Toxicity data from humans: other indications*

RIF has also been used at higher doses for other mycobacterial indications and for a wide range of non-mycobacterial infections. These experiences support the contention that the vast majority of RIF's adverse effects are idiosyncratic, and not dose related.[66-72] Details are provided in section 17.4.3.

2.3.1.4 *Toxicity of companion drugs*

Information on toxicity of companion drugs is summarized below and elaborated in section 17.4.4.

Isoniazid

The incidence of all adverse effects from isoniazid is approximately 5%, many of which do not require discontinuation of the drug. Peripheral neurotoxicity is dose dependent and uncommon at conventional doses (<0.2%). The risk of peripheral neuropathy is increased by malnutrition or co-morbidity. Pyridoxine (vitamin B_6) prophylaxis is recommended for these persons, and will be given to all patients in this trial.

Pyrazinamide

The most frequent pyrazinamide effects are skin rash, gastrointestinal intolerance, hepatotoxicity (1.3%), arthralgias (1-7%), hyperuricemia due to blockage of urate excretion (up to 66%), and rarely acute gouty arthritis.[73, 74] These side effects are seldom dose-limiting. Asymptomatic elevations in serum uric acid are frequent but do not generally require treatment or discontinuation of TB therapy.[75, 76] Minor arthralgias can usually be treated with non-steroidal anti-inflammatory agents while continuing the drug. The most common serious side effect of pyrazinamide is hepatotoxicity. In two randomized clinical trials the

addition of pyrazinamide to isoniazid and rifampin did not increase the rates of hepatotoxicity above that seen with the latter two drugs alone.[76, 77] However, three recent retrospective cohort studies suggest that the incidence of pyrazinamide-induced hepatitis during active TB treatment is higher than that for other first-line TB drugs, and higher than previously recognized.[78-80] Although PZA hepatotoxicity is clearly dose-related, there is no evidence that increased RIF doses, in combination with PZA, INH, and RIF, are likely to increase the incidence of PZA-related hepatotoxicity.[81] The elevated frequency of hepatotoxicity with RIF/PZA appears to be idiosyncratic and has only been observed in the absence of INH (and EMB).

Ethambutol

Ethambutol is usually well-tolerated with low rates of skin rash, nausea, vomiting, or diarrhea. Fever, allergic reactions, abdominal pain, mental status changes, peripheral neuropathy, and increased liver function tests have rarely been associated with ethambutol. Adverse events occur in less than 2% of patients receiving ethambutol at the 15 mg/kg dose and include decreased visual acuity (0.8%), rash (0.5%) and asymptomatic hyperuricemia.[73] The most common serious side effect of ethambutol is retinal toxicity, often first perceived as a decrease in color perception. Patients receiving ethambutol should be instructed about symptoms of ocular toxicity. If stopped promptly, permanent visual loss is rare among patients with ethambutol-related retinal toxicity. Rates of retinal toxicity are very low when the drug is given for relatively short periods of time, as is the case in this study.

2.3.1.5 *Drug-drug interactions with RIF*

Drug classes and members with reported interactions with RIF are described in Table 2. This includes drugs for which potential drug interactions or synergistic hepatotoxicity are a known concern, such as boosted protease inhibitors, non-nucleoside reverse transcriptase inhibitors, azole antifungals, statins. Suggestions for the management of rifampin-related drug interactions likely to be relevant in the study site are also presented. A prominent drug interaction of rifampin is that involving hormonal contraceptives. Women of childbearing potential (i.e., not surgically sterilized or postmenopausal for less than 1 year) will be advised to use a double-barrier contraceptive method including the following: condom, intravaginal spermicide (foams, jellies, sponge), and diaphragm; cervical cap; or intrauterine device.

Table 2 *Drug-drug interactions with RIF (likely in HIRIF setting) requiring change of medication*

Drug class	Drugs affected by the rifamycins	Comments
Anti-infectives	HIV protease inhibitors	Only antiretroviral therapy containing efavirenz with two nucleosides or a triple-NRTI regimen should be used by study subjects
	Macrolide antibiotics	Azithromycin has no significant interaction with rifamycins. Clarithromycin and erythromycin should not be used
	Doxycycline	May require use of an alternate drug or drug combination (e.g., azithromycin)
	Atovaquone	Consider alternate form of *Pneumocystis carinii*

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

		treatment or prophylaxis (e.g., pentamidine)
	Chloramphenicol	Consider an alternative antibiotic (e.g., 3rd-generation cephalosporin)
	Mefloquine	Consider alternate form of malaria treatment or prophylaxis (e.g., primaquine)
Hormone therapy	Ethinylestradiol, norethindrone	Women of reproductive potential on oral contraceptives should add a double-barrier method of contraception
Immuno-suppressive agents	Cyclosporine, tacrolimus	These drugs should not be used with rifampin. Consider alternate treatment (e.g., sirolimus)

Ethanol can exacerbate the potential hepatotoxicity of isoniazid, rifampin, and pyrazinamide. Participants will be urged to abstain from alcohol while on study phase therapy.

Table 3 *Drugs with potential drug interactions or synergistic hepatotoxicity effect*

Medication Class	Names (brand name in parenthesis)
Boosted protease inhibitors	Saquinavir/ritonavir or any of the following PIs in combination (boosted) with ritonavir; Tipranavir (Aptivus), indinavir (Crixivan), saquinavir (Invirase), lopinavir+ritonavir (Kaletra), fosamprenavir (Lexiva), ritonavir (Norvir), darunavir (Prezista), atazanavir (Reyataz), nelfinavir (Viracept)
Non-nucleoside reverse transcriptase inhibitors	Rilpivirine (Edurant), etravirine (Intelence), Delavirdine (Rescriptor), efavirenz (Sustiva), nevirapine (Viramune)
Systemic Azole antifungals	Fluconazole (Diflucan), hexaconazole, itraconazole, posaconazole, voriconazole
Statins	Atorvastatin (Lipitor, Torvast), fluvastatin (Lescol), lovastatin (Mevacor, Altocor, Altoprev), pitavastatin (Livalo, Pitava), pravastatin (Pravachol, Selektine, Lipostat), soruvastatin (Crestor) and simvastatin (Zocor, Lipex)

Table 4 *Antibiotics contraindicated during study's treatment*

Abbreviation	Drug
Amk	Amikacin
Amxc	Amoxicillin/Clavulanate
Cfx	Ciprofloxacin
Cfz	Clofazimine
Cm	Capreomicyn
Clr	Clarithromycin
Cs	Cyclocerine
Eto	Ethionamide
Gfx	Gatifloxacin
Gem	Gemifloxacin
Km	Kanamycin
Lfx	Levofloxacin
Lzd	Linezolid
Mzl	Metronidazole
Mfx	Moxifloxacin
Ofx	Ofloxacin
Pas	p-aminosalicylic acid
Pto	Prothionamide
Rbt	Rifabutin
Rpt	Rifapentin
Spr	Sparfloxacin
S	Streptomycin
Th	Thioacetazone
Trd	Terizidone
Vi	Viomycin
Micobacterial drugs under investigation	OPC-67683 y TMC 207

2.3.1.6 *Drug interactions between RIF and companion drugs*

Since INH, PZA, and EMB are metabolized via independent metabolic pathways, there do not appear to be any clinically significant drug interactions between RIF and the companion drugs proposed for this study.[82-86] However, since the potential for changes in the pharmacokinetics of INH, PZA, and EMB when given with higher doses of RIF has not been entirely ruled out, we will also assess the effect of the increased RIF dose on the concentrations of the three companion TB drugs. Additional details are provided in section 17.4.5.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

2.3.1.7 *Potential interactions between companion medications and concomitant drugs*

The companion drugs, INH, PZA and EMB, may also result in interactions with concomitant drugs. Implicated classes include anti-convulsants, sedatives, neuroleptics, anticoagulants, narcotics, anti-infectives, anti-gout, and antacids, in addition to several others. Details of the class members with interactions with companion drugs and suggestions for their management are listed in Section 17.4.6.

2.3.1.8 *Other Risks and Discomforts*

Home Visits

While study participant information is completely confidential and private, there is a risk that a participant's health status may become known to others through study team visits to the participant's home. Everything possible will be done to prevent any type of discrimination towards the participant. If members of the study team are allowed by the participant to visit their home, study workers will not identify themselves as health workers, nor will they dress the part (such as wearing a white jacket).

Blood Draws

There will be several blood samples collected from the participant throughout the duration of the study. Risks of a blood draw may include pain or bruising where the needle is inserted, redness, fainting, or, in rare cases, an infection. While most sample collection requires only one puncture, more than one may be necessary.

HIV/Viral Hepatitis (B & C) Testing

HIV and Hepatitis B & C testing both incorporate risks of a blood draw. There is also the possibility of discrimination against individuals with HIV/Hepatitis B or C that may cause them to feel badly when they learn about their infection. The study team will do everything possible to reduce these risks. If diagnosed with Hepatitis B or C, the study team will direct the patient toward appropriate care, including facilitating access to the Peruvian national insurance program. If HIV infection is diagnosed, facilitated referrals will be made to the national STD, HIV/AIDS program. Under this program, the Ministry of Health provides free treatment for HIV, so the benefits of knowledge of HIV status are greater than the risks mentioned.

Confidentiality

There is a possibility of discrimination against individuals taking part in this study as medical information becomes known. Information collected from study participants during the study will be completely private and confidential. Precautionary measures are taken to make sure that patient confidentiality is upheld, including keeping patient information in private, locked files in the study office and having information on the computer be confidential, password protected, encrypted or protected by other security measures. Additionally, personal information will be kept confidential; only the study ID number will be used to identify the patient by the study team.

Genetic Testing

Genetic testing incorporates risks of a blood draw. This study will test only for specific markers that are associated with increased risk of adverse events; markers that are not likely to result in any discrimination even if they do become known. Participants will have the option to provide a blood sample for genetic

testing. The results of the study that uses the genetic testing data will be made available to the participant if they wish.

Chest X-Ray

Each chest x-ray will expose the patient to a low amount of radiation that is similar to the amount of radiation an average person is exposed to from the sun over the course of 10 days. It is also possible that while doing a chest x-ray, something that looks like a medical problem other than tuberculosis may be seen. If the radiologist thinks there may be a problem, s/he will tell the patient and help him/her receive follow-up care. However, if the doctor thinks the patient might have an additional medical problem, and s/he does not, it may cause unnecessary worry.

2.3.2 Potential Benefits

High-dose RIF could increase the frequency of cure, reducing transmission and morbidity and mortality due to TB. This intervention could also result in decreased selection for RIF-resistant mutations. Moreover, if inclusion of high-dose RIF results in treatment shortening, program resources can be extended to permit treatment of additional patients. Lastly, completion of this trial will contribute to strengthening scarce clinical trials capability worldwide.[87]

2.3.2.1 *Benefits to study participants*

All subjects enrolled will receive an anti-tuberculosis regimen of six months duration containing INH, PZA, EMB, and RIF, which, at standard RIF doses, is reported to cure approximately 95% of patients treated under trial conditions. The treatment provided in the intervention arms may or may not have activity that exceeds the standard treatment used in the control arm. Symptoms of TB and infectiousness may or may not resolve more quickly in these subjects. They may or may not be able to return to activities of daily life sooner as well. Subjects in all three arms will receive TB care that is of similar or superior quality to that received routinely through the public sector in the study site. Moreover, subjects will receive necessary support to facilitate completion of the study regimen.

2.3.2.2 *Potential impact on health care, policy or practice*

The proposed study will provide essential information that may support the development of a shorter, effective treatment regimen for the 9 million people who develop TB annually. Demonstrably increased sterilizing activity of an anti-TB agent, without increased adverse events, would indicate a larger study of a shortened regimen. Since RIF is available globally, a shortened regimen, containing RIF, could be integrated into national TB programs. Its expected impact on the global TB burden[88] could be dramatic, potentially achieving one or more of the following: 1) reducing the probability of default, relapse, and/or resistance by shortening treatment;[89, 90] 2) decreasing the cost of treatment; 3) increasing the number of patients who could be served by TB programs, by decreasing the resources required for each patient; 4) reducing transmission; if the sputum of patients on high-dose RIF is sterilized more quickly than that of patients on conventional doses of RIF, additional transmission may be averted.

The second broad benefit of this trial derives from its importance for other TB drug development projects. Subjects treated for eight weeks will be followed until the end of treatment and for six additional months. Consequently, we will also be able to contribute to the evaluation of bacteriologic indicators of treatment response for possible use as surrogate endpoints in Phase II and phase III trials of TB therapy. These trials have typically relied on failure/relapse as the primary endpoint. Phase III trials are long, with final results not available until at least one year after treatment completion. Identifying early predictors of treatment

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

failure and relapse will have substantial implications for the cost and duration of future clinical trials of anti-TB agents.

The potential impact of this study is heightened by plans to coordinate efforts with a complementary trial, which began enrolling in 2011 in Africa (ClinicalTrials.gov identifier: NCT00760149). This study is not being conducted under IND. Protocols and study procedures for the proposed trial in Latin America and this study are being harmonized, facilitating pooling of the data. Prior to this pooling, a statistical analysis plan will be jointly developed. The benefits would be multiple: substantially more data would be available to evaluate both the safety and efficacy endpoints, adding increased certainty around effect estimates. Heterogeneity in efficacy or toxicity between different populations could be observed. Genotypic differences as well as differences in frequency of co-infection could be important between the two study populations.

3.0 STUDY OBJECTIVES & OUTCOME MEASURES

3.1 Study Objectives

Across three, daily, oral doses of RIF (10, 15 and 20 mg/kg/day):

3.1.1 Primary

1. To assess the difference in steady state pharmacokinetic exposure of RIF and 25-desacetyl-rifampin.
2. To assess the difference in sputum culture sterilization during the initial 8 weeks.
3. To compare the incidence of adverse events related to the study drug or regimen during the initial 8 weeks of daily four-drug treatment, and up to four weeks later.

3.1.2 Secondary

1. To estimate the relationship between pharmacokinetic parameters and measures of sputum culture sterilization.
2. To compare 4 methods for estimating sterilizing activity: 1) decline in log colony-forming units (CFUs), 2) speed of culture conversion in solid & liquid media, 3) increased time to detection in liquid medium, and 4) proportion of patients with positive cultures at 8 weeks.

3.2 Study Outcome Measures

3.2.1 Primary

1. $AUC_{0\text{-}24}$/MIC of RIF at steady state.
2. Serial decline in *M. tuberculosis* colony counts in the sputum (Log_{10} CFU/mL/day).
3. Incidence of adverse events related to study drug or regimen, grade 2 or higher, during the 8-week intensive phase, and up to four weeks after.

3.2.2 Secondary

1. Association between $AUC_{0\text{-}24}$/MIC (and alternative measures such as $AUC_{0\text{-}6}$) and bacteriologic endpoints (log_{10} CFU/mL/day, time to conversion, time to detection, negativity at 8 weeks).
2. Association among log_{10} CFU/mL/day, time to sputum culture conversion on solid and liquid media; time to detection of *M. tuberculosis* in liquid medium; frequency of sputum culture negativity on solid medium at eight weeks.

4.0 STUDY DESIGN

This is a Phase II dose-ranging trial comparing 3 doses of RIF in a multidrug regimen for treatment of smear-positive, pulmonary TB. Patients will be randomized to 3 arms, in a 1:1:1 allocation.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

The intervention phase of this prospective, randomized, triple-blinded trial will last 8 weeks, the duration of the standard "intensive" phase for short-course chemotherapy for TB. During that time, subjects will receive the following companion drugs (weight-based doses are specified below in Table 5): isoniazid (INH, 5 mg/kg/day), ethambutol (EMB, 20 mg/kg/day), and pyrazinamide (PZA, 25 mg/kg/day), pyridoxine (50 mg), the standard weight-based doses used in treatment. Subjects will also be randomized to receive one of the following weight-based doses of the study drug, rifampin (RIF): 10 mg/kg/day (standard dose, control), 15 mg/kg/day (intervention 1), 20 mg/kg/day (intervention 2). Placebo will be used to control only the additional RIF capsules provided in the intervention arms. Subjects, clinicians, and laboratory staff will be blinded to study arm. All patients in the same weight band will receive the same total number of tablets (fixed-dose combination, dosed according to package inserts plus RIF and/or placebo). They will also all receive 50 mg of pyridoxine three times per week to prevent peripheral neuropathy, a common side effect of INH.

All participants will receive a 4-month continuation phase of thrice weekly INH (10 mg/kg) and RIF (10 mg/kg) per WHO recommendations[91] as well as pyridoxine 50 mg three times per week.

4.1 Study Population

The study population will comprise adults (>=18 years and <=60 years) with newly diagnosed, previously untreated, smear positive (>=2+) pulmonary tuberculosis presenting in metropolitan Lima. Recruitment will take place in the following health districts (DISA, Spanish acronym): DISA IV-Lima Este (including health network Lima Este Metropolitana) and DISA V-Lima Ciudad (including the health networks Rímac-San Martin de Porres-Los Olivos-Túpac Amaru and Lima) where the local study partner, Socios En Salud, has established relationships. If necessary, recruitment may be extended to additional health networks or facilities , located in convenient proximity to the study hospitals, with approval of local authorities.

In 2010, the Lima Este Metropolitana health network, reported 1160 cases of smear-positive, pulmonary TB. Approximately 4% of TB cases were co-infected with HIV and roughly 10% also had diabetes mellitus.

In 2011, the Rímac-San Martin de Porres-Los Olivos health network reported 785 cases of smear-positive pulmonary TB. Approximately 3% of those cases had HIV-coinfection. The Túpac Amaru health network registered 611 cases that same year; 2% of TB patients were co-infected with HIV.

Patients from included facilities, who are identified as potential study participants, will be referred to either Hospital Nacional Hipólito Unanue or Hospital Nacional Sergio E. Bernales according to their jurisdiction. Both hospitals are INS-certified Research Centers.

Recruiting in both Lima Este and Lima Ciudad, we estimate that we will randomize approximately 17 patients per month. This would result in randomization of 180 participants, the study target, in approximately -11 months.

4.2 Treatment Delivery and Duration

Study drug and companion drugs will be delivered 7 days/week during 8 weeks. However, participants may take as long as 9 weeks to complete the required 56 doses. If one or more of the study drugs is suspended for more than two weeks, the participant will be withdrawn from the study. All study participants will receive WHO-recommended continuation therapy (INH 10 mg/kg/day and RIF 10 mg/kg/day) 3 days/week for 18 weeks and pyridoxine 50 mg 3 days/week. All doses in the intensive and

continuation phases will be directly observed. ;. Routine, monthly bacteriologic monitoring will continue during this period.

Post-treatment follow-up will be 6 months from ingestion of last dose of directly observed therapy. Total participation time for each subject will be 12 months. The trial will last 18 to 24 months.

4.3 Assessment of Study Objectives & Measurement of Endpoints

Primary and secondary outcome measures are enumerated in sections 3.2.1 & 3.2.2.

4.3.1 Centralization of Evaluations

All clinical evaluations, adverse event (AE)/adherence assessments, and pharmacokinetic (PK) sampling will all occur at one of the hospitals, Hospital Nacional Hipólito Unanue or Hospital Sergio E. Bernales. Each patient's study visits will take place at the hospital most conveniently located relative the patient's home. Overnight pooled sputum collection will also take place at the hospitals, or when staffing and infection-control conditions permit, in participants' homes. One dedicated research microbiology laboratory will perform all of the study TB microbiology. Routine hematology and blood chemistry will be performed in a single private reference lab in Peru. For unscheduled laboratory tests, the study may also use the hospital laboratories.

Site investigators will evaluate chest x-rays for inclusion criteria. One independent pulmonologist will read and score all chest x-rays; scores will be used as a covariate in analysis.

4.3.2 Pharmacokinetic-Pharmacodynamic Endpoints

Strong evidence presented in section 2.2.1.3 and section 17.3.3 supports the concept of exposure-related pharmacodynamics for RIF with a PK-PD index parameter of AUC/MIC. $AUC_{0\text{-}24}$/MIC has therefore been selected as the primary PK endpoint for this study. $AUC_{0\text{-}24}$, $AUC_{0\text{-}6}$, C_{max}, T_{max} of RIF and 25-desacetyl-RIF will be derived from measurements of RIF and 25-desacetyl-RIF in plasma samples drawn from participants after at least 13 days of treatment (after steady state is achieved). Plasma concentrations for the companion drugs will also be measured using the same sampling scheme as for RIF. This information will be important for adjusted analysis of the relationship between concentration and activity and will provide pharmacokinetic data on anti-TB drugs in South American populations. This complements existing data on Asian and South African populations.[7, 24, 30, 92] Bioanalysis will be performed using a validated high-performance liquid chromatography method in Dr. Peloquin's lab. Additional details are in protocol sections 8.3 and 8.4.

RIF minimum inhibitory concentrations (MICs) will be measured in a pre-treatment sputum specimen collected from each study participant. The assays will be performed according to guidance from the Clinical Laboratory Standards Institute,[93] evaluating 10 concentrations of rifampin, including the 1 mg/liter "critical concentration" of RIF. The assay will be performed at the SES Laboratory in Peru. On a small sample of patient isolates (4 in each arm), MICs of 25-desacetyl-RIF will also be measured.

4.3.3 Bacteriologic endpoints

As outlined in section 2.1 and section 17.1 there is currently no universal agreement as to the best surrogate endpoint for assessment of sterilizing activity. Although there is reasonable historical support for the use of solid culture at two months as a surrogate for relapse, this endpoint lacks power even when comparing distinct regimens. It is, therefore, probably not feasible for use in dose-ranging studies.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Recently rate-based measures using more intensive sputum bacteriology during the first two months have also been used for this purpose and promise improved efficiency of early clinical evaluation.[94, 95] However, whether solid or liquid culture methods should be used and whether they should be analyzed using a time-to-event or non-linear regression approach remains unclear. Hence most clinical studies currently incorporate both bacteriological methods and more than one analysis approach with a view to subsequent evaluation of the utility of these different approaches. The non-linear regression approach to quantitative sputum culture on solid selective media has been selected as the primary endpoint for this study since two independent studies appear to confirm its ability to detect sterilizing activity[95, 96] and meaningful statistical comparisons will be possible at the sample size selected for the PK study proposed. Nevertheless, three secondary bacteriological endpoints will also be obtained for the purposes of comparison with the non-linear regression results and as a contribution towards evaluation of their relative support.

The decline in log colony forming units will be calculated based on measurements from pooled sputum samples collected at specified time points during the first 2 months of treatment. (The details of the sampling scheme are specified in section 7.2.1.3 and in the schedule of events, Figure 2). Each patient will provide samples on day 0 and on 5 other days. This sampling scheme represents the best balance among burden on study participants, power to detect a difference, and accuracy of the estimate. Samples will be cultured on selective, solid 7H11 (Middlebrook) medium.

Time to culture conversion will be calculated as the interval between the start of treatment and the date of collection of sputum, which, when cultured in LJ medium, results in the first observed negative sputum culture in a profile terminating in negative cultures. A culture can be read as negative only after 60 days.

Change in time to positivity will be measured as the rate at which the difference between date of inoculation and date of observed growth for specimens cultured in the MGIT (liquid) system changes with time.

Frequency of culture conversion at two months will be measured as a count of study participants whose sputum culture (grown in solid LJ medium), collected after 2 months of treatment, is negative after 60 days of incubation.

4.3.4 Safety endpoints

Section 2.3.1 (and section 17.4) describes the known toxicities associated with RIF and their attribution to higher doses of RIF. Of particular interest for ultimate implementation of a TB regimen with higher doses of RIF are toxicities so severe or dangerous as to result in discontinuation of treatment, in particular hepatitis and hematologic disorders.

The primary endpoint for the analysis of safety and tolerability *will be the incidence of adverse events grade 2 or higher, related to the study drug or regimen, occurring during the 8-week intensive phase*.

Secondary safety and tolerability endpoints include the frequency and timing of Grade 3 and 4 toxicities that are determined by the investigators to be related to study drug or regimen.

The *frequency and timing of permanent discontinuation* for any reason other than being found to be ineligible for continuing in the study (because of drug-resistance, or lack of growth of *M. tuberculosis* from the initial sputum culture) will also be recorded for secondary analyses.

Adverse events will be measured through clinical and laboratory evaluations at intervals specified in section 7.0. Please see Table 9 for modified study-related toxicity criteria. The adverse events will be graded according to Table 9, which is derived from the DMID Adult Toxicity Tables (Draft November

2007) (see *Figure 8* in section 17.6). The interval from treatment start until the event is first observed will be recorded.

4.3.5 Safety monitoring committee (SMC)

An SMC will be formed and will function according to DMID guidelines (http://www3.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/safetyoversight.htm). The SMC will comprise one clinical TB expert, one internationally recognized clinical trialist, and one clinical researcher with expertise in a topic of relevance to the study (e.g., hepatotoxicity), none of whom will be otherwise involved in the study. A statistician may also participate, either as a full voting member or as a consultant to the SMC at open session meetings. S/he will not otherwise be involved in the study. Additional expertise may be added for open or closed meetings, as needed, on an ad hoc basis. Open meetings will be held after 60 patients have completed 4 weeks of study treatment and then again after 120 patients have completed 4 weeks of study treatment or as needed for SAEs and unexpected AEs. If the committee determines that access to unblinded data is required, the meeting(s) will be closed executive session(s). Please see section 9.6 for more detail regarding safety oversight.

4.3.6 Interim analysis plans

An early safety evaluation will be performed when the first 60 patients (approximately 20 per arm) have completed 4 weeks of study treatment. Safety data will be reviewed by the SMC. The SMC will provide recommendations for further action, including continuation of all three arms, discontinuation of one arm (with rollover to standard dose of RIF or lower intervention dose), or termination of the trial. In addition, the SMC will review trial progress after 120 participants have completed 4 weeks of study treatment.

An interim, blinded PK analysis will be performed by Dr. Davies when PK data are available for half the patients.

No interim efficacy analysis is planned for this Phase II trial.

4.4 Substudies

Substudies will contribute to the knowledge base of the microbiology and molecular biology of *M. tuberculosis*. Our understanding of several microbiologic endpoints will be refined through this trial. Through three substudies, we will examine: 1) the value of lipid-body content in sputum samples as a potential surrogate marker for clinical endpoints; 2) the effect of decontamination on early- and late-phase quantitative culture results, and the agreement between days to positivity (DTP) in MGIT and SSCC in both early and late phases; and, 3) questions of emerging importance about mixed strain (or super) infection: what is the frequency of mixed strain infections detectable in pooled sputum collections? What is the contribution of mixed strain infections on interim and clinical endpoints?

4.4.1 LipTox Red Staining Study design

4.4.1.1 *Aim*

To evaluate the utility of LipTox Red staining as a measure of response of persister sub-populations to therapy and as a predictor of treatment success.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

4.4.1.2 *Objectives*

1) To describe the proportion and estimated absolute number of LipTox Red-positive organisms in sputum at baseline

2) To estimate changes in the proportion and estimated absolute number of LipTox Red-positive organisms during the course of therapy

3) To relate measures of LipTox Red positivity at baseline to late phase decline in colony counts derived from non-linear mixed effects analysis of quantitative culture on solid media

4) To relate measures of LipTox Red positivity at baseline to changes in DTP in automated liquid culture

4.4.1.3 *Participants*

Participants in the study will already have been recruited to the HIRIF study. The three sputum samples for this substudy are all samples that will already be collected during the HIRIF study and are already described in the HIRIF study protocol.

4.4.1.4 *Procedures*

The early-morning sputum samples collected for the study at baseline, at study visit 5 or 6 (see Figure 2. Schedule of Events) and at visit 7 will be used for smear microscopy on site and for assessment of lipid body content in Liverpool. Frozen sputum samples will be sent to University of Liverpool for processing, reading, and analysis.

4.4.1.5 *Analysis*

The proportion and number of LipTox Red-positive cells will be summarized graphically and expressed as mean or median with appropriate confidence or interquartile intervals and ranges. Exploratory data analysis will relate these findings to other covariates obtained in the study such as baseline colony count, DTP, and radiological extent of disease.

The analysis of colony counting and liquid culture data from the HIRIF study will employ a multivariate mixed effects approach with a bi-exponential or non-parametric structure as appropriate. The proportion of LipTox Red-positive cells will be evaluated as a covariate in these models using Wald tests of the coefficients and the likelihood ratio test to assess overall model fit. Other relevant covariates to be included in the model will be HIV status, radiological extent of disease, and dose size of rifampin.

The profiles of estimated absolute numbers of LipTox Red-positive cells will also be modeled using a mixed effects approach with an appropriate structural model to be determined from the data since no longitudinal data yet exists. A linear contrast for treatment effect will be fitted to the final model.

4.4.2 Variability in early- and late-phase activity by microbiological procedures

4.4.2.1 *Aim*

To estimate whether the effect of sputum decontamination on colony counts obtained on selective solid medium is the same at all stages of treatment.

4.4.2.2 *Objectives*

1) To compare the colony counts determined on selective solid medium to those determined on decontaminated, selective solid medium at each sputum sampling point.

2) To compare colony counts on selective solid medium determined using both methods (with decontamination and without decontamination) with DTP at each sputum sampling point.

3) To estimate rates of contamination of cultures on selective solid medium according to method of sputum processing.

4.4.2.3 *Participants*

Participants in the study will already have been recruited to the HIRIF study. No additional samples or clinical study procedures will be required in addition to those specified by the HIRIF protocol.

4.4.2.4 *Procedures*

In the Lima study lab, each of the pooled sputum samples (of sufficient volume), collected at 6 time points during study treatment, will be cultured in three different ways: (1) 1/3 of the specimen will be digested with dithiothreitol (DTT), but not decontaminated, and plated out from serial dilutions on selective 7H11 plates for colony counting; this is considered the "standard" method; (2) 1/3 of the specimen will be decontaminated with the NALC procedure followed by plating out from serial dilutions on selective 7H11 plates for colony counting; (3) 1/3 will be decontaminated with the NALC procedure and cultured in MGIT liquid medium bottles.

4.4.2.5 *Analysis*

The mean profiles of colony counts and the confidence intervals obtained using both methods of sputum processing will be plotted over all the sampling points. Summary statistics representing the slope of the decline of colony counts in the time intervals 0-2, 2-14 and 14-56 days will also be computed and the effect of decontamination assessed using analysis of variance for each time period. A linear or generalized additive model will also be used to relate the DTP results in liquid culture to colony counts on solid medium and to examine the effect of the covariates, day of sample, and sputum processing method on the regression coefficients of this model.

4.4.3 Complex infection in pooled sputum samples

4.4.3.1 *Aim*

To evaluate the use of pooled sputum samples to identify complex infections and understand the impact of such infections on treatment response.

4.4.3.2 *Objectives*

1) To assess the pre-treatment prevalence of complex infections identifiable in pooled sputum specimens and

2) To measure the impact of complex infection measured from pooled sputum on early treatment response.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

4.4.3.3 *Participants*

Participants in this substudy will be accrued from the study population recruited for the HIRIF study. No additional samples or clinical study procedures will be required in addition to those specified by the HIRIF protocol.

4.4.3.4 *Procedures*

Genotyping will be performed to determine if, within each specimen collected, more than one molecularly distinct strain (representing either multiple strain infection or with-in host diversification of a single infecting strain) can be identified.

4.4.3.5 *Analysis*

The proportion of infections with evidence of clonal heterogeneity or multiple strains will be measured at each sampling point (stratified by treatment arm). Genotyping will also permit identification of strain lineage.

5.0 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Study Population

HIV-infected and -uninfected individuals 18 to 60 years with newly diagnosed, previously untreated, smear-positive (>= 2+), pulmonary TB may be evaluated for eligibility.

We will randomize 180 subjects. Sufficient statistical power for the primary endpoint will be retained as long as no more than 30 patients are lost to follow up. Therefore, we expect at least 150 subjects, 50 in each arm, to complete the study treatment and follow up.

Approximately 40% of subjects will be female, while 60% will be male. This reflects the relative frequency of case notification between men and women in the study site (57% of cases notified in Peru in 2007 were male).[1]

Patients will be recruited from ambulatory facilities in the Peruvian public health system in the DISA IV-Lima Este and in the DISA V-Lima Ciudad. These health centers function as part of a network of centers with personnel dedicated to TB screening, diagnosis, and treatment. Patients with a single positive sputum smear (>=2+) will be invited to participate by health center staff and then recommended for screening. Interested patients will be referred to study staff at the two Hospital Research Centers.

5.2 Recruitment and Retention

Recruitment will occur over an 11-month period. In light of the enrollment targets relative to the number of incident cases of TB, the investigators do not expect difficulties enrolling subjects through the routine passive case detection services in operation at the study sites. Recruitment progress will be continually reevaluated to determine if additional sites or intensified case-finding will be necessary to meet recruitment goals.

Retention will be assured through a system of DOT supervisors and enablers. All treatment will be ambulatory, and delivered by dedicated DOT supervisors in health centers or in a location of each subject's choosing (e.g., workplace, home). During the intensive phase of the study, treatment adherence will be confirmed through bi-weekly medication review visits. In the continuation phase, treatment adherence will be confirmed through monthly visits. Transport costs for study visits, as well as for collection of PK and sputum samples, will be covered by the study. Participants will receive food vouchers monthly during the intensive and continuation phases, and quarterly during follow-up. Meals will also be provided during inpatient stays for pooled sputum collection and PK sampling.

5.3 Subject Inclusion Criteria

Inclusion criteria for the trial are:

1. Newly diagnosed pulmonary TB with acid-fast bacilli (>=2+) in a stained sputum smear.
2. Susceptibility of isolate to INH and RIF by HAIN test.
3. Willingness to undergo HIV testing according to the National Health Guidelines for TB control in Peru. The study will also consider patients who have had negative HIV serostatus documented within six months prior to enrollment or if verifiable positive serostatus was documented using a validated test any time previously.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

4. Age ≥ 18 years and <61 years.
5. Signed informed consent.
6. Negative serum pregnancy test (women of childbearing potential).
7. Women of child-bearing potential must agree to practice a double-barrier method of birth control during treatment. Adequate contraceptives (condoms and spermicide) will be provided by the study to avoid pregnancy among female subjects.
8. Karnofsky score of at least 50 (requires considerable assistance and frequent medical care).
9. Intends to remain in jurisdiction of health center during study and follow up.

5.4 Subject Exclusion Criteria

Subjects will be excluded from the trial for any of the following:

1. Body weight <30 kg.
2. Prior treatment with multidrug anti-TB therapy for more than one month.
3. Resistance on HAIN to INH and/or RIF. These patients will be treated according to local programmatic guidelines.
4. Central nervous system or miliary TB.
5. Clinical or radiological signs suggestive of pericardial or pleural involvement.
6. Presence of significant hemoptysis. Patients who cough up frank blood (more than blood-streaked sputum) will not be eligible for enrollment.
7. Known intolerance to any of the study drugs. Use of concomitant drugs that interfere with the pharmacokinetics of anti-TB drugs (see Table 2); use of concomitant hepatotoxic drugs (other than companion study drugs) for which potential drug interactions or synergistic toxicity are known: boosted protease inhibitors, non-nucleoside reverse transcriptase inhibitors, azole antifungals and statins (see Table 3); use of antibiotics that are contraindicated during the study's TB therapy (see Table 4). Current daily use of acetaminophen or paracetamol for two weeks or more.
8. History of liver disease.
9. Uncontrolled condition that might interfere with drug absorption, distribution, metabolism or excretion (i.e. chronic gastro-intestinal disease, renal insufficiency defined by creatinine clearance <60mL/min).
10. Uncontrolled diabetes mellitus (HbA1c>7.5%).
11. Refusal to be tested for HIV infection; HIV infection with contraindication for treatment with efavirenz (including resistance).
12. Pulmonary silicosis.

13. Breastfeeding.
14. Rifampin contraindications such as hypersensitivity or jaundice.
15. Likely difficulty adhering to the study protocol, as assessed by the investigator.
16. Laboratory results in the 14 days preceding enrollment showing:
    a. Serum amino alanine transferase (ALT) >2 times upper limit of normal
    b. Serum total bilirubin concentration >2.5 times upper limit of normal
    c. Serum creatinine concentration > 2 times upper limit of normal and/or creatinine clearance <60 mL/min
    d. Hemoglobin concentration < 7.0 g/dL
    e. Platelet count < 150,000/mm^3
    f. White blood count <4500 cells/µL.
17. Having a serological test positive for HBVsAg (hepatitis B virus surface antigen) or for HCVAb (hepatitis C virus antibody) test.

5.5 Treatment Assignment Procedures

This will be a triple-blinded (participants, clinical staff, and laboratory staff) randomized trial. Treatment assignment will be randomized by the study statistician.

5.6 Randomization Procedures

Eligible patients will be randomized to 3 arms, in a 1:1:1 allocation. Randomization to RIF dose will be implemented by the pharmacist at the study site using a predetermined randomization scheme created by the study statistician at the study data and coordinating center (DCC) at Harvard University. Randomization will be blocked. Randomization will not be stratified.[97]

5.7 Blinding Procedures

Participants, clinicians, and laboratory staff will be blinded to treatment assignment. Blinding of clinicians and laboratory staff is essential to reduce the probability of biased reporting of adverse events and laboratory endpoints, respectively. To protect blinding, block size will not be available to investigators. Pharmacy staff will not be blinded to assignment. Pharmacy staff will receive randomization assignment from the DCC and prepare medication boxes according to patient weight and treatment-arm assignment. Pharmacy staff will have no contact with participants and will not communicate treatment assignment information to clinical or laboratory staff. Subjects across all 3 arms within a single weight band will receive identical numbers of capsules (RIF and/or placebo) and FDCs (see Table 5).

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

5.8 Withdrawal

5.8.1 Reasons for withdrawal

Subjects may withdraw voluntarily from participation in the study at any time upon request. Subjects may also withdraw voluntarily from receiving the study intervention for any reason.

In addition to voluntary withdrawal, study drugs may be discontinued by trial staff for the following reasons:

- Any clinical AE or laboratory abnormality that, depending on its nature and severity, requires temporary suspension of the study drugs until the toxicity resolves as indicated in the subsequent section describing adverse event management (Section 9.2.3). If one or more of the study drugs is suspended for more than two weeks, the participant will be withdrawn from the study.
- An intercurrent illness, other medical condition or situation occurs such that continued administration of study drugs and/or participation in the study would not be in the best interest of the subject.
- Development of any exclusion criteria may be cause for study withdrawal.
- Any patient without culture confirmation of *M. tuberculosis*, or whose isolate is found to be resistant to any of the study drugs after study treatment is initiated, will be discontinued from the study, but followed for 14 days to detect late toxicities from study therapy. If a participant's isolate tests resistant to ethambutol or pyrazinamide, in the absence of resistance to isoniazid or rifampin, confirmation of the resistant result will be required prior to termination from the study.

The subject will continue to be followed for 14 days with the subject's permission if withdrawal occurs. If a subject becomes pregnant while receiving therapy and study drugs are discontinued, the newborn will be followed for six months.

In the event of study closure by the sponsor or regulatory agency, study subjects on intensive phase TB treatment will be discontinued from their assigned study regimen. Subjects will be treated with a standard regimen for TB according to local guidelines.

5.8.2 Handling of Withdrawals

Attempts to contact participants who do not complete study follow-up will include at least 3 home visits and 1 telephone call, if appropriate. Interviews with patients who withdraw from the study will focus on the reason for discontinuation and whether it was related to development of adverse reactions. If withdrawal occurs, the subject will be asked to continue scheduled evaluations.

5.8.2.1 *Procedures/Evaluations in case of withdrawal*

If withdrawal occurs, and we have the participant's permission, subjects will be given appropriate care under medical supervision until the symptoms of any AE resolve or stabilize. Subjects still receiving TB treatment will be referred to the local TB clinic or hospital outpatient TB program for care.

If a participant withdraws prior to completion of two weeks of study drug, the patient will be included only in analysis of safety. Since blood samples will not have been collected for PK, participants

withdrawing before two weeks will not be included in PK analysis. And, an insufficient number of sputum samples will have been collected for inclusion in the efficacy analysis.

If a participant withdraws after completion of two—but before completion of eight—weeks of study therapy, s/he will be included in the safety and PK analyses; available SSCC data will also be used in the efficacy analysis.

If a participant withdraws after completion of eight weeks of study therapy, s/he will be included in safety, efficacy, and PK analyses.

If the subject is willing, a termination visit will be performed.

5.9 Termination of Study

The study may be terminated by DMID, the PIs, one of the IRBs, the FDA or another regulatory agency. Termination could occur after the development of unacceptable toxicities or as a recommended outcome of an SMC review.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

6.0 INVESTIGATIONAL PRODUCT

6.1 Study Product Description

RIFADIN (rifampin capsules USP) for oral administration contain 150 mg rifampin per capsule. The capsules also contain, as inactive ingredients: corn starch, D&C Red No. 28, FD&C Blue No. 1, FD&C Red No. 40, gelatin, magnesium stearate, and titanium dioxide.

Rifampin is a semisynthetic antibiotic derivative of the natural product rifamycin SV. Rifampin is a red-brown crystalline powder very slightly soluble in water at neutral pH, freely soluble in chloroform, soluble in ethyl acetate and in methanol. Its molecular weight is 822.95 and its chemical formula is C43H58N4O12. The chemical name for rifampin is either:

3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin

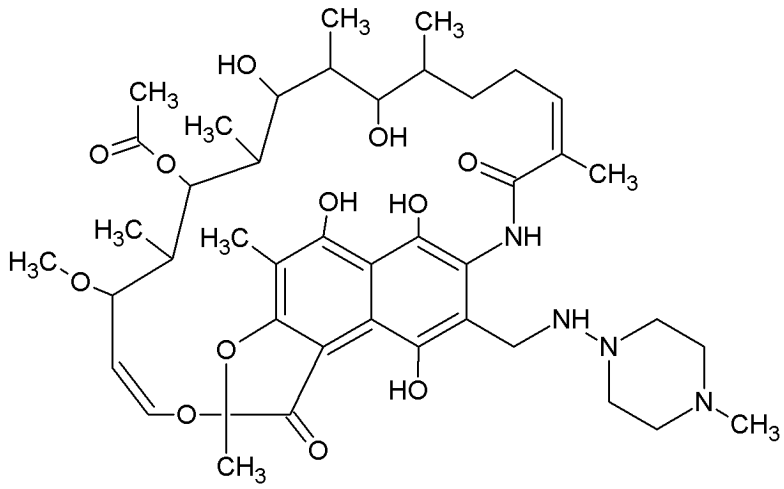
Or

5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,20,22–

heptamethyl-8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-1,11(2H)-dione 21-acetate.

Its structural formula is:

Figure 1. Chemical structure of rifampin



6.2 Companion drugs

6.2.1 Ethambutol

Ethambutol (EMB) is an ethylenediimino derivative of butanol that interferes with cell wall synthesis in mycobacteria; other bacteria are uniformly resistant to EMB. EMB's primary contribution to anti-TB

treatment is to prevent the emergence of drug resistant strains. EMB has no sterilizing activity at clinically-tolerated doses.[98]

6.2.2 Isoniazid

Isoniazid (INH) is the hydrazide of isonicotinic acid and is one of the primary drugs for tuberculosis treatment. Its spectrum of activity is limited to the mycobacteria of the *M. tuberculosis* complex; it is bactericidal for rapidly replicating organisms and bacteriostatic for dormant bacilli. INH is thought to act by inhibiting biosynthesis of mycolic acids, which are a component of the mycobacterial cell wall.

6.2.3 Pyrazinamide

Pyrazinamide (PZA) is an analog of nicotinamide and has unique activity against *M. tuberculosis*. In liquid culture media, PZA has little activity except at low pH. However, in animal models and in the treatment of human tuberculosis PZA has unique sterilizing activity; its addition to the multidrug regimen permitted treatment duration to be decreased from 9 months to 6 months, when used in conjunction with RIF. The mechanism of action of pyrazinamide remains unknown.

6.2.4 Placebo

The scarlet 150 mg placebo capsules contains, as inactive ingredients: corn starch, D&C Red No. 28, FD&C Blue No. 1, FD&C Red No. 40, gelatin, magnesium stearate, and titanium dioxide.

6.3 Adjunctive therapies

6.3.1 Pyridoxine

Pyridoxine is a member of the water soluble B vitamin group. Vitamin B6, or pyridoxine, is active in the metabolism of proteins, carbohydrates and fats. It is also a necessary part of hemoglobin synthesis. B6 deficiency results in retarded growth and a peripheral neuropathy. All patients will receive 50 mg of pyridoxine three times per week during the study's intensive and continuation phase.

6.4 Acquisition of Study Drugs

Study drug and placebo will be donated by Sanofi aventis, 82 Ave Raspail, 94256 Gentilly Cedex, FRANCE. Companion drugs (FDCs) and pyridoxine will be purchased from MacLeod pharmaceuticals.

6.4.1 Quality Assurance of Study Drugs

The study will be performed under US FDA IND (#106635). The study drug is approved by a stringent regulatory authority (EMA). MacLeod, the company from which companion FDC tablets will be procured, has been pre-qualified by the World Health Organization's Global Drug Facility.

6.4.2 Formulation, Packaging, and Labeling

In the intensive phase of treatment, Rifadin and placebo will be supplied in blister packs of 14 150-mg blue and scarlet capsules. Standard Rifadin doses and companion drugs will be supplied in FDCs. Pyridoxine will be supplied in bottles containing 6 50-mg tablets.

In the continuation phase of treatment, Rifadin will be supplied in blister packs of 12 150-mg blue and scarlet capsules. Isoniazid will be supplied in bottles containing 100 mg tablets, the number of tablets in

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

each bottle will be according to patient's weight band. Pyridoxine will be supplied in bottles containing 12 50-mg tablets.

Package inserts for Rifadin, FDCs, and pyridoxine are included in section 17.5.

6.4.3 Product Storage and Stability

Rifadin and companion drugs should be kept tightly closed, in a dry place, away from excessive heat according to the manufacturer'. The storage facility will be kept locked. Access to the storage facility will be limited to the study pharmacy team. Visitors to the facility will be recorded in a study log. Lastly, inventory will be controlled and logged by pharmacy staff.

6.5 Dosage, Preparation and Administration of Investigational Product

To simplify treatment administration, companion drugs will be administered in FDCs. Since all FDCs contain RIF, participants will receive standard weight-based dosing of FDCs, including RIF at approximately 10 mg/kg (maximum of 750 mg in >70 kg participants, per FDC package). In the intervention groups, this will be supplemented by loose capsules of 150 mg each to reach doses of approximately 15 (maximum of 1200 mg in >70 kg participants) or 20 mg/kg (maximum of 1500 mg in >70 kg participants). Participants in the control group will receive placebos in numbers to match the active capsules received by intervention subjects in the same weight band (see Table 5). All administration will be oral and directly observed, once daily, seven days/week, for eight weeks.

Table 5 *Dosing of RIF by treatment arm and baseline weight*

r=150 mg RIF p=150 mg placebo			**Control group**		**Intervention 1**		**Intervention 2**	
Weight bands	**# of FDCs**	**RIF from FDCs**	**# RIF & placebo**	**Total RIF**	**# RIF & placebo**	**Total RIF**	**# RIF & placebo**	**Total RIF**
30-37	2	300	0r + 2p	300	1r + 1p	450	2r + 0p	600
38-54	3	450	0r + 2p	450	1r + 1p	600	2r + 0p	750
55-70	4	600	0r + 4p	600	2r + 2p	900	4r + 0p	1200
>70	5	750	0r + 5p	750	3r + 2p	1200	5r + 0p	1500

6.5.1 Modification of Investigational Product for a Participant

Certain events or conditions may necessitate modification or temporary or permanent discontinuation of the study medication. Patients who experience such events or conditions, however, will still be "on study" and will be followed until study completion. Any patient for whom the study medication is temporarily discontinued will be restarted on study medication as soon as possible as their clinical condition dictates. Study regimens will be discontinued and non-study regimens will be used with continued study follow-up for patients in whom treatment-emergent drug toxicity warrants discontinuation of study therapy and for patients who become pregnant. If study drugs are permanently discontinued, further antituberculosis therapy may be administered at the local clinical investigator's discretion in coordination with the local TB program. These patients will be followed in the study for a maximum of 6 months of TB treatment, according to the guidelines and time-points established in the protocol, starting from the date of initiation of HIRIF intensive phase treatment.

6.5.1.1 *Criteria for temporary discontinuation of study drugs*

- Any clinical AE or laboratory abnormality that, depending on its nature and severity, requires temporary discontinuation of the study drugs until the toxicity resolves as indicated in the subsequent section describing adverse event management (Section 9.2.3).
- An intercurrent illness, other medical condition or situation occurs such that continued administration of study drugs and/or participation in the study would not be in the best interest of the subject.
- Development of any exclusion criteria may be cause for discontinuation.

6.5.1.2 *Criteria for permanent discontinuation of study therapy*

- Development of a toxicity that warrants permanent discontinuation of any study drug (refer to section 9.2.3).
- The patient refuses further therapy or withdraws for any reason.
- It is the investigator's judgment that it is no longer in the best interest of the patient to continue study therapy.
- Termination of the study.

If a patient refuses further therapy or withdraws consent for treatment under the protocol, the patient will be treated with a non-study regimen. If the patient has withdrawn consent, all follow-up will stop.

6.6 Accountability Procedures for the Investigational Product

The investigator will acknowledge receipt of the study drug, companion drugs, and matching placebos. The study pharmacist will maintain inventory of the study drug, companion drugs and matching placebos. Study staff has the responsibility to assure that study drugs are dispensed to patients in compliance with the protocol.

6.7 Assessment of Subject Compliance with Investigational Product

Administration of all doses of study and companion drugs will be supervised by study staff during the intensive and continuation phases. . Delivery of study medications may occur in the health center, hospital, or a place convenient to the subject (home, place of work). The decision will be made jointly between subjects and treatment supervisors. Adherence to study drug and companion drugs will be assessed and captured on a case report form (CRF) at visits where used drug supply is returned and new drug supply is dispensed.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

6.8 Concomitant Medications/Treatments

Companion drugs will also be administered orally in FDC once daily, seven days/week for eight weeks. Standard weight-based dosing will be used (as illustrated in Table 6). Additionally, all patients will receive 50 mg of Pyridoxine three times per week.

Table 6 *Doses of companion medications during the intensive phase*

Drug	Dose for daily therapy
Isoniazid	
30-37 kg	150 mg
38-54 kg	225 mg
55-70 kg	300 mg
> 70 kg	375 mg
Pyrazinamide	
30-37 kg	800 mg
38-54 kg	1200 mg
55-70 kg	1600 mg
> 70 kg	2000 mg
Ethambutol	
30-37 kg	550 mg
38-54 kg	825 mg
55-70 kg	1100 mg
> 70 kg	1375 mg

In the continuation phase, all patients will receive 4 months (18 weeks) of RIF 10 mg/kg/day and INH 10 mg/kg/day thrice weekly according to WHO guidelines. For RIF, patients weighing 30-37 kg will receive no more than 300 mg/day. Patients weighing 38-49 kg will receive a maximum of 450 mg/day, and patients weighing >=50 kg are not to exceed 600 mg/day.

For INH, patients weighing 30-39 kg will receive no more than 300 mg/day. Patients weighing 40-49 kg will receive a maximum of 400 mg/day, patients weighing 50-59 kg will receive a maximum of 500 mg/day, patients weighing 60-69 kg will receive a maximum of 600 mg/day, and patients weighing =>70 kg are not to exceed 700 mg/day.

In the continuation phase, patients will also receive 50 mg of Pyridoxine three times per week.

7.0 STUDY SCHEDULE

This is summarized in the Schedule of Events (Figure 2) and detailed below.

7.1 Screening/Enrollment

7.1.1 Screening for eligibility, day -3 to 0

Any patient presenting to a participating health center with at least one positive sputum smear (>=2+) will be asked by health center staff about their interest in participating. Study personnel will then explain the study, including the screening process. The patient will receive a consent form and be encouraged to consider participation in consultation with family. No more than 48 hours after study staff becomes aware of a positive sputum smear result (>=2+), study personnel will regain contact with the prospective participant. Those willing to participate will be consented by a study physician. If the patient consents, the following screening procedures will be implemented beginning on screening day-3.

1. Record demographics including age (Confirm 60>= age >=18 years), gender, and race.

2. Complete physical exam including neurologic assessment, weight, vitals, Karnofsky assessment; rule out extrapulmonary TB through clinical screening.

3. Complete medical history and concomitant medication review per checklist.

4. HIV screening according to the National Health Guidelines for the TB control in Peru. Potential participants found to be HIV positive will receive a facilitated referral to the National STD HIV/AIDS program for confirmatory testing and treatment.

5. Collect 1 sputum sample for smear, and if positive (>1+) HAIN test, and culture. Results of HAIN test must be available before initiation of study therapy. The strain isolated from this specimen will also be used for MIC measurement.

6. Perform posterior-anterior chest x-ray; rule out miliary TB, pleural or pericardial involvement. If a chest x-ray has been performed and documented within 2 weeks prior to enrollment, and is made available by the patient to the study, and is of quality judged to be adequate by a site PI or Sub-I, it will be used in lieu of a new x-ray. This will reduce participant exposure to radiation.

7. Draw blood samples (approximately 10 ml) for hematology (CBC with differential), biochemistry (LFTs, albumin, alkaline phosphatase), creatinine, HbA1c, Hepatitis B & C serology and serum pregnancy tests (if female). Results must be available before initiation of study therapy. Any abnormal results, which do not meet the threshold for exclusion, will be recorded as baseline findings. A portion of the blood sample collected on day -3 will be saved for pharmacogenetic testing, which will be performed only on samples from patients who meet all inclusion criteria.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Figure 2. Schedule of Events

						Intensive Phase							Continuation Phase				Follow up		
						Month 1					Month 2								
PROCEDURES	Screening	Pre-Dosing Day 0				Week 1			Week 2	Week 4	Week 6	Week 8	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12- Final Study Visit	Early Term. Visit[1]
Visit	1	2	3[2]	3[2]	4[3]	4[3]	5	5	6	7	8	9	10	11	12	13	14	15	ET
Day	-3 to 0	0	1	2	3	7	8[4]	9[5]	14	28	42	56-64[6]	90	120	150	182	270	365	
Informed Consent	X																		
Demographics	X																		
Physical Exam	X								X	X	X	X	X	X	X	X	X	X	X
Neurologic Exam	X								X	X	X	X							
Weight	X		X						X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X		X						X	X	X	X	X	X	X	X	X	X	X
Karnofsky Score	X											X						X	X
Medical History	X																		
Overnight Pooled Sputum Collection[7], by collection group		1, 2[8]	1[9]	2[9]	1	2	1	2	1[10]	2[11]	1[12]	2[13]							
Early Morning Sputum Collection, Smear Microscopy	X[14]							2[15]	1[13]	X[13]		X[16]	X	X	X	X	X	X[14]	X[14]
Culture Solid Medium	X									X		X	X	X	X	X	X	X	X
DST		X[17]												X[18]					X[16]
PK Sampling, scheduled by sputum collection group									1[19]	2[17]									

						Intensive Phase							Continuation Phase				Follow up		
						Month 1					Month 2								
PROCEDURES	Screening	Pre-Dosing Day 0				Week 1			Week 2	Week 4	Week 6	Week 8	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12- Final Study Visit	Early Term. Visit[1]
Visit	1	2	3[2]	3[2]	4[3]	4[3]	5	5	6	7	8	9	10	11	12	13	14	15	ET
Day	-3 to 0	0	1	2	3	7	8[4]	9[5]	14	28	42	56-64[6]	90	120	150	182	270	365	
Chest Radiograph	X											X				X			X[20]
HIV Screening	X											X							X[20]
Complete Blood Count with Differential	X								X	X		X							X[18]
Blood Chemistry[21]	X								X	X	X	X							X[18]
Creatinine	X									X		X							
HbA1c	X																		
Pregnancy Screening (β-HCG)	X																		
Height			X																
Evaluation Criteria Inclusion – Exclusion			X																
Randomization			X																
Acuity & Color Vision Test[22]			X									X							X[18]

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

						Intensive Phase							Continuation Phase				Follow up		
						Month 1					Month 2								
PROCEDURES	Screening	Pre-Dosing Day 0				Week 1			Week 2	Week 4	Week 6	Week 8	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12- Final Study Visit	Early Term. Visit[1]
Visit	1	2	3[2]	3[2]	4[3]	4[3]	5	5	6	7	8	9	10	11	12	13	14	15	ET
Day	-3 to 0	0	1	2	3	7	8[4]	9[5]	14	28	42	56-64[6]	90	120	150	182	270	365	
Hepatitis B & C Serology	X																		
Pharmacogenetic Testing	X[23]																		
Urine Specimen, Proteinuria (scheduled by sputum collection group)			X						1	2		X							X[18]
Assessment of AE's & Drug Toxicity Counseling		X	X		1	2	1	2	X	X	X	X	X	X	X	X	X	X	X
New Supply of Study Medication & Companion Drugs			X						X	X	X	X	X	X	X				
Study & Companion Drug Adherence Assessment									X	X	X	X	X	X	X	X			
Interval Medical History[24]					1	2			X	X	X	X	X	X	X	X	X	X	X

Notes:

'1' indicates procedures in which only sputum collection Group 1 will take part.
'2' indicates procedures in which only sputum collection Group 2 will take part.
'X' indicates procedures in which all study participants will take part.

---

[1]The elements listed here are for early termination during the continuation phase. If early termination occurs during the intensive phase, the visit should include the procedures listed as well as chest x-ray; complete blood count, blood chemistry; visual acuity and color vision testing; and urinalysis.
[2] All participants will also receive integrated care, according to National TB Guidelines, including a psychological, nutritional and social work evaluation. Patients with diabetes will also have an evaluation with an endocrinologist who will determine diabetes management and follow-up.
[3] Visit 4 can take place anytime between Day 3 and Day 7. However, Visit 4 sputum collection should not fall on the night immediately succeeding the sputum collection for Visit 3, i.e., for a given participant, there should be at least one night with no collection between Visit 3 and Visit 4 overnight collections
[4] Can take place between days 8-10.
[5] Can take place between days 9-11.
[6] Patients who had one or more of the study drugs suspended and then returned to full dose, may have until treatment day 72 to complete the intensive phase of the study.
[7] MGIT culture and quantitative culture will be performed on all overnight, pooled samples.
[8] In addition to quantitative culture, samples will be used for MIC.
[9] Overnight pooled sputum collections for groups 1 and 2 at Visit 3 can take place on either Day 1 or Day 2.
[10] Can take place between days 14-16.
[11] Can take place between days 26-30.
12 Can take place between days 40-44.
[13] Can take place between days 54-66.
[14] One sample is collected. HAIN test will be performed on positive smears. Specimens with *M. tuberculosis* sensitive to RIF and INH on HAIN will be cultured on L.J. medium. A subculture will be plated in Middlebrook and used to measure minimum inhibitory concentrations.
[15] Store samples to be used for LipTox
[16] Two samples should be collected for smear microscopy
[17] DST will be performed only if sensitive to H and R on HAIN test; DST to be performed in MGIT
[18] DST will be performed only on the first culture that is positive, at or after Month 4 and continuing through Month 12.
[19] PK visit will take place the morning following the fifth overnight sputum collection visit, on day 15th or 29th
[20] These procedures will only be done, in addition to those listed, if early termination occurs during the intensive phase
[21] AST, ALT, ALP, bilirubin, albumin.
[22] Repeated in case of reported loss of visual acuity or color vision.
[23] Pharmacogenetic testing will be performed on blood collected at study visit 1, only among those who meet all study inclusion criteria after screening is completed.
[24] Includes review of concomitant medications, signs and symptoms that might have appeared since last visit, according to body organ and system.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

7.1.2 Pre-Dosing/treatment initiation

The initiation visit should begin no more than 3 days after pre-screening, among those established as eligible.

Pre-dosing, day 0 (visit 2):

Procedures or activities not completed during the screening visit will be completed on this day.

One overnight, pooled sputum sample collection begins at the pre-dosing visit and ends the next day, on study treatment initiation day.
Conventional susceptibility testing to confirm rapid INH & RIF sensitivity, as well as to test susceptibility to PZA & EMB, will be performed in MGIT on the strain isolated from this first overnight sputum sample. In case this sample is not adequate we will use the morning sputum sample.

Treatment initiation, day 1 (visit 3):

The following activities will take place:

1. Measurement of vital signs: heart rate, blood pressure, respiratory rate, and weight, and height.
2. Complete Snellen visual acuity examination & Ishihara color test.
3. Urinalysis (proteinuria).
4. Toxicity counseling and counseling on potential failure of hormonal contraception and alternative methods. The study will provide subjects with condoms and spermicide to avoid pregnancy among female participants.
5. All participants will receive standard integrated care, according to National TB Guidelines, including a psychological, nutritional, and social work evaluation. Patients with diabetes will also have an evaluation with an endocrinologist who will determine the patients' corresponding diabetes management and follow-up.
6. Review of inclusion and exclusion criteria.
7. Randomization assignments will be made to: treatment group (1:1:1); PK group (sparse or intensive sampling, 2:1); and pooled sputum sample collection schedule (1:1).
8. First dose of treatment
9. 2-week supply of study medication and companion drugs will be provided at treatment initiation.

A log will be maintained of persons who are eligible but do not initiate study treatment.

7.2 On-treatment

7.2.1 Intensive Phase

7.2.1.1 *Treatment support*

Study DOT supervisors will be trained nursing assistants or lay workers with commensurate experience. They will be trained in GCP, including for the administration of anti-TB medication and in triage for signs or symptoms of adverse events. They will observe administration of treatment daily, in the patient's home, health center, or other elected location. DOT supervisors will record dose administration in the Treatment Supervision Card. Supervisors will be in daily contact with study nurses and will report any signs or symptoms of toxicity to study nurses, who will determine if an unscheduled visit is necessary.

7.2.1.2 *Clinical and laboratory follow-up*

1. Visit at **week 1** will include an interval medical history as well as Adverse Event & Treatment adherence assessment. Participants will be asked about what medications they have been taking and if they have had any illnesses since the last visit.

2. Visits at **weeks 2, 4, & 6** will comprise an interval medical history, physical and neurological exam, including vital signs and weight. Study participants will also receive a new supply of medications for the subsequent 2 weeks, according to weight band and treatment assignment. All doses will be supervised by DOT supervisors. Participants will be asked about what medications they have been taking and if they have had any illnesses since the last visit. Adherence to study medications since the previous visit will be documented.

3. Additionally, visits at **weeks 2 & 4** will comprise of the drawing of blood samples for CBC with differential and chemistry (AST, ALT, ALP, bilirubin, albumin). Creatinine will be measured at week 4. Any new abnormal findings, or changes from baseline, will be considered AEs: they will be graded, assessed for relation to study drug, determined as expected or unexpected, and reported according to regulatory requirements.

4. **Week 6 visit** will comprise a complete physical and neurological exam, including weight, vital signs and interval medical history. Participants will be asked about what medications they have been taking and if they have had any illnesses since the last visit. Blood draw for chemistry will be performed.

These visits should occur within 2 days (before or after) the actual week's end.

7.2.1.3 *Bacteriologic follow-up*

As noted above, participants will be assigned randomly to one of two pooled sputum collection schedules. Dividing the participants into two groups, each providing ½ the required samples, permits optimization of the number of time points at which bacteriologic specimens will be available, while reducing the burden on study participants. Half the participants in each study arm will be assigned to each sample schedule. This is illustrated in Table 7, where an "X" denotes that participants in the specified sputum collection group will provide an overnight pooled sputum sample beginning on the specified day at one of the inpatient clinical research facilities (the TB ward at either Hospital Nacional Hipólito Unanue or Hospital Sergio E. Bernales in Lima) or at the participant's home. All overnight sample collections will be supervised by a DOT supervisor or study nurse. The sample will be used for assessment of microbiologic

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

endpoints. On certain days, as noted in the Schedule of Events (Figure 2) an additional early-morning sputum sample will be required for smear microscopy and LJ culture.

Sputum will not be induced in cases when spontaneous expectoration is not successful. This is not standard practice in the study site, nor has induction been used in published SSCC studies. Moreover, the accuracy of calculation of CFU decline is unlikely to be compromised by a few, inevitable missing results. Information from all pooled sputum samples from all participants will be used to calculate decline in colony forming units. Plans for missing bacteriologic data are detailed in the analysis section.

Table 7 *Overnight, pooled sputum collection suggested schedule by assigned group*

Treatment Day[1]	**0**	**1**	**2**	**3**	**7**	**8**[2]	**9**[2]	**14**[2]	**28**[3]	**42**[3]	**56**[3]
Visit Number	**2**	**3**		**4**		**5**		**6**	**7**	**8**	**9**
Sample #	**1**	**2**		**3**		**4**		**5**		**6**	
Group 1	x	x		x		x		x		x	
Group 2	x		x		x		x		x		x

7.2.1.4 *Pharmacokinetics, [d13-56]*

After no fewer than 13 and no more than 56 (including 3 consecutive daily) doses of RIF and companion drugs administered by DOT, patients will be admitted to a study research center for PK sampling. The preference is to draw the PK blood samples at the 5th overnight sputum sample collection in order to minimize the number of patient visits. PK sampling, however, may occur anytime between treatment days 14 and 56.

Participants will arrive at the clinical research center the night before the scheduled draw. Participants will be weighed; urine and sputum samples will be collected, and vital signs measured. In the morning, a standard breakfast will be provided for all subjects no less than 4 hours prior to or 2 hours after treatment administration (details will be provided in the PK procedures).

Study treatment will be administered and observed in the clinical research center with time of dosing recorded on the patient's medical record. Subjects will abstain from eating (may drink water) for 2 hours after the time of treatment administration.

Similar to the bacteriology scheme, efficiencies in sampling are being achieved by randomly assigning participants within arms to two groups, 2:1 sparse to intensive sampling. Subjects randomized to the sparse sampling scheme (2/3 of participants) will have blood samples drawn for PK at the following fixed time points: 0, 120, and 360 minutes after dosing (10 ml at each time point).

Subjects randomized to the intensive sampling scheme will have blood samples drawn for PK at any time during the following sampling windows 0, 15-45 mins, 45-75 mins, 75-105 mins, 105-135 mins, 345-375 mins, and 825-855 mins after dosing (10 ml at each time point).

Further details about PK sampling can be found in section 8.3.1.

[1] Sputum collection will be completed on the day specified in the table, having started the night before.
[2] Window: Up to 2 days after.
[3] Window: Up to 2 days before or 2 days after.

7.2.2 End of intensive phase study visit

The end of the intensive phase of therapy is defined by number of doses (56) and should be completed no later than **treatment day 64**. Patients who had one or more of the study drugs suspended and then returned to full dose, may have until treatment day 72 to complete the intensive phase of the study. The end of intensive phase study visit will comprise a physical exam, including weight, vital signs, Karnofsky assessment. A neurological exam will be performed to monitor for peripheral neuropathy. Chest radiograph will be performed. Blood samples will be drawn for HIV testing, CBC with differential, blood chemistry, and creatinine, visual acuity and color vision testing will be repeated, as will urinalysis. Patients will be asked as part of the interval medical history about what medications they have been taking and if they have had any illnesses since the last visit. Any new abnormal findings, or changes from baseline, will be considered AEs: they will be graded, assessed for relation to study drug, determined as expected or unexpected, and reported according to regulatory requirements. Adherence to study medications since the previous visit will be documented. A new 1-month supply of study medication will also be given.

In addition, two sputum specimens will be obtained to assess sputum-culture and -smear conversion. These specimens must be obtained on the day of completing the intensive phase of therapy or up to one week after completion, but before more than one dose of continuation therapy has been given. The two sputum specimens can be obtained on the same day; at least one should be early morning.

7.2.3 Continuation Phase

7.2.3.1 *Clinical and laboratory follow-up*

Patients will have study visits 3, 4, 5, and 6 months after treatment initiation (these should occur within one week of the target date). They will comprise a physical exam, including weight and vital signs, and interval medical history. At each study visit during the continuation phase of therapy, patients will be asked what medications they have been taking and if they have had any illnesses since the last visit. At each of these visits, participants will have their adherence to study medications since the previous visit documented. At months 3, 4, and 5, participants will receive a new supply of medication.

Patients will be assessed for delayed toxicity from the intensive phase of therapy. This will consist of a symptom-driven examination. Laboratory testing will be at the discretion of the lead study clinician. At the 3-month visit, any new abnormal findings, or changes from baseline, will be considered AEs: they will be graded, assessed for relation to study drug, determined as expected or unexpected, and reported according to regulatory requirements.

7.2.3.2 *Bacteriology*

An early morning sputum sample will be obtained at 3, 4, 5, and 6 months for smear microscopy and LJ culture (samples must be taken no less than 26 days and no more than 35 days after the previous sample). A sputum sample will be defined as unobtainable (and negative for analytic purposes of the binary endpoints) if no sputum can be obtained.

Repeat DST will be done only on the first positive culture occurring at or after the 4- month visit. Patients with resistant isolates will be referred for alternative treatment according to local protocol. These patients will be included in PK, efficacy, & safety analyses. All patients with positive bacteriology at month 5 or 6 will be assigned outcomes of treatment failure.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

The continuation phase will end after 54 doses of continuation-phase therapy have been administered. The final on-treatment bacteriology and clinical visits will occur no later than one week after treatment has been completed.

7.2.4 Follow-up period

At months 9 & 12 (visits to occur not less than 12 weeks or more than 16 weeks after the prior visit) follow-up visits will be made. Visits will comprise a physical exam, including weight, vital signs, and medical history.

Patients will have an assessment for delayed toxicity from the intensive phase of therapy. This will consist of a symptom-driven examination. Laboratory testing will be at the discretion of the clinical investigators. Patients will be screened for active tuberculosis, based on signs and symptoms. Early morning sputum samples will be collected for sputum smear microscopy and culture in solid medium. Repeat DST will be done only on the first positive culture occurring during follow up. Patients with resistant isolates will be referred for alternative treatment according to local protocol. Positive bacteriology in the follow-up period will result in classification of treatment outcome as recurrence. Molecular fingerprinting will be performed on the recurrent isolate to compare to the baseline isolate. Patients will be asked about what medications they have been taking and if they have had any illnesses since the last visit.

7.3 Final Study Visit

The visit at month 12, or no more than 30 weeks after treatment completion, will represent the final study visit. This will comprise physical exam with: weight, vital signs, Karnofsky assessment, and interval medical history. Two early-morning sputum samples will also be collected and tested by smear microscopy and culture in solid medium. Repeat DST will be done on any culture, newly positive for *M. tuberculosis* (i.e., negative throughout the follow-up period, but positive at final visit). Patients with resistant isolates will be referred for alternative treatment according to local protocol. Positive bacteriology at the final study visit will result in classification of treatment outcome as recurrence. Molecular fingerprinting will be performed on the recurrent isolate to compare to the baseline isolate.

7.4 Early Termination Visit

If termination occurs during the continuation phase, then the early termination visit will include the following:

Physical exam, weight, vital signs, Karnofsky assessment, assessment of adverse events, and interval medical history (inquiry about what medications they have been taking and if they have had any illnesses since the previous visit) will be performed. Two early-morning sputum samples will also be collected for smear microscopy and culture on LJ. Repeat DST will be done on any culture, newly positive for *M. tuberculosis* (i.e., negative throughout the continuation period, but positive at early termination visit). Patients with resistant isolates will be referred for alternative treatment according to local protocol. Positive bacteriology at the final study visit will result in classification of treatment outcome as recurrence. Molecular fingerprinting will be performed on the recurrent isolate to compare to the baseline isolate.

If termination occurs during the intensive phase, then the visit will include the elements described above as well as: chest x-ray, complete blood count, blood chemistry; visual acuity and color vision testing; and urinalysis.

7.5 Unscheduled Visit

Unscheduled visits in patient homes, health centers, or research centers may occur for the following reasons, among others: adverse events occurring between scheduled study visits, patient perception of non-response to study medication, difficulty with adherence. Follow up AE/SAE management procedures are detailed in sections 9.2.3 and 9.5.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

8.0 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Study DOT supervisors will accompany study participants at all study visits.

Medical history will be obtained from patient interview with research center staff at initial screening. History will involve establishing occurrence and timing of any prior TB disease. Questioning will ascertain history of all medications taken, including anti-TB drugs. Prospective participants will be asked if they are currently taking any of the drugs in Table 2 (list of contraindicated medications) or if they have previously taken any treatments that would indicate underlying disease, which precludes participation. Interview will inquire about HIV testing/status, pregnancy, breastfeeding, and other conditions that could result in ineligibility (i.e., pulmonary silicosis, chronic gastro-intestinal disease, liver disease, uncontrolled Diabetes Mellitus or renal disease), alcohol or drug abuse. Karnofsky evaluation will also be performed.

Physical examination will comprise: vital signs (measurement of blood pressure, respiratory rate, temperature, peripheral pulse), height (only at treatment initiation visit) and weight and review of organ systems (respiratory, cardiovascular, gastro-intestinal, musculoskeletal, neurologic).

I**nterval histories** will ascertain history of medications taken since last history, including anti-TB drugs, and any adverse events experienced. Where possible, identification of the causative agent will be attempted. Participants will be asked if they are currently taking any of the drugs in Table 2 (list of contraindicated medications). Interview will inquire about pregnancy, breastfeeding, and other conditions that would result in ineligibility, as well as about alcohol or drug abuse.

Counseling procedures. Study participants will be advised by study nurses of the importance of completing all doses of treatment, in spite of mild discomforts. They will be informed by study nurses of possible adverse events (dizziness, somnolence, joint pain, pruritis, erythema, discoloration of urine, tears, perspiration; increased appetite, anorexia/hyporexia, nausea/vomiting, abdominal pain, jaundice). Symptoms reported at the time of supervised treatment will be recorded and managed according to protocol section 9.2.3.

8.2 Laboratory Evaluations

8.2.1 Hematology and chemistry

Hematology will include complete blood count with differential, including platelets. Chemistry tests will comprise a full liver profile including AST, ALT, ALP, bilirubin, and albumin. These will provide lab values to assess the development of important adverse events--hepatotoxicity, thrombocytopenia, leucopenia--which may be associated with high-dose RIF.

At baseline, samples will be taken also for HbA1c, ß-HCG, and creatinine; potential participants with uncontrolled diabetes (HbA1c>7.5), creatinine clearance <60mL/min, or who are pregnant will be excluded. Hepatitis B&C serology will be performed on a blood sample collected at the screening visit. Those patients with positive HBVsAg or HCVAb will be excluded from study participation.

During the whole study, we will collect approximately 80 to 120 mL of blood from study participants.

Biochemistry and hematology assays will be performed at a private, clinical research lab in Lima, Peru.

8.2.2 Bacteriology

Bacteriology procedures are summarized here. All study microbiology procedures will conform to standards specified by the Clinical Laboratory Standards Institute.

8.2.2.1 *Serial sputum colony counts and days to positivity*

Pooled sputum samples will be collected from all patients at 6 time points during the intensive phase of treatment (as specified in Table 7). Patients will be in a hospital research center or supervised in their home overnight for approximately 12 hours, assisted by a study nurse or nursing assistant, to provide continuous sputum samples in sterile wide-mouthed plastic container (honey jar approximately 200-250 ml volume container) and stored on ice for the duration of the collection. Although attention will be paid to assure the quality and quantity of samples (as described in lab procedures), there will be no minimum sample volume requirement.

Samples will be transported by study staff to the bacteriology lab. Transport will require maintenance of the cold chain with an insulated pack and ice, which are separated from the specimen by a divider.

Processing and testing will be performed in the BSL 3 lab, using at least BSL 2 practices and procedures. Samples will be divided into three parts for "standard" & decontaminated plating in 7H11 medium and for MGIT culture. There is no minimum sputum sample size required for processing. After the 1st part is taken, there may be smaller quantities left for the 2nd & 3rd methods (especially later in treatment, when sputum production is often low). To assure that the smallest amounts are not always used for the same method, the order of assignment of parts to each microbiologic assay will be randomized according to the sputum sample distribution procedure.

A sample of sputum specimens will be sent to SGUL on not more than 4 occasions to check SSCC enumerations of viable colony counts. No more than 50 specimens of 1 ml each—from sputum samples of at least 3 ml volume—will be shipped for this purpose. The technique used at SGUL will be the same as is used in the Lima study lab.

8.2.2.2 *Smear, culture, sensitivity tests, and RIF MICs*

Smear, culture, and sensitivity testing will be performed at the intervals specified in the schedule of events, Figure 2 and section 7.0. All of these assays will be performed at the dedicated study lab in Lima.

i. Direct smear examination (DSE)

At each sampling point, participants will provide an early-morning sputum sample. Direct smear examination (DSE) will be performed on all sputum specimens. These include use of new slides; with approximately 10µl-30µl of sputum spread over an area of 2 cm by 1cm. Sputum will be dried and fixed (avoiding excessive heat). Staining will be either Ziehl Neelsen or Auramine and examined as quickly as possible after staining, rinsing, and air-drying. Auramine-stained slides will be read first by fluorescence examination with a 25X objective. Situations may require confirmation by Ziehl-Neelsen (100X objective) or a second reader. Semi-quantitative results will be recorded.

ii. Mycobacterial culture in solid medium

Samples will be cultured on LJ slopes in the BSL 3 lab, using at least BSL 2 practices and procedures. Sputum will be digested and decontaminated using NALC-NaOH, with exposure to decontaminant

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

limited to avoid killing mycobacteria. The specimen will be centrifuged, the supernatant decanted, and the sediment neutralized prior to inoculation on the medium. All cultures will be examined 72 hours after inoculation to detect contaminants; thereafter, cultures will be examined weekly for 8 weeks. Growth rate determination and colony morphology will be used as well as the niacin test for identification of mycobacteria. Semi-quantitative results will be reported.

iii. RIF MICs

RIF MICs will be measured in positive cultures grown on 7H11 medium from the pre-treatment sputum specimen. This will be performed at the SES microbiology lab either immediately after collection or in batches, after storage at -70 Celsius. The 7H11 medium has been selected for SSCC and MICs as it contains enzymatic casein hydrolysate, which promotes the growth of a few strains of *M. tuberculosis* that grow with difficulty or not at all on 7H10.[99] Performing the assay on two samples permits distinction between intra- and inter-patient variability in RIF MICs. MIC will be measured by inoculating each strain of *M. tuberculosis* onto sectors of 7H11 agar medium in a series of 10 plates, containing the concentrations of RIF specified in Table 8.

Table 8 *Concentrations of rifampin in plates with their log values*

Plate No	RIF µg/ml	Log RIF
1	0.000	
2	0.088	-1.054
3	0.125	-0.903
4	0.177	-0.753
5	0.250	-0.602
6	0.354	-0.452
7	0.500	-0.301
8	0.707	-0.151
9	1.000	0.000
10	1.414	0.151

The plates will be divided into sections (8 or fewer) to permit testing of multiple specimens on a single day. Plates will be incubated for 4 weeks in the incubator and classified as growth or no growth. Plates will be reviewed from highest to lowest RIF concentrations and the concentration of the last slope with no growth will be the minimum inhibitory concentration.

8.2.3 Drug susceptibility testing (DST)

HAIN GenoType MTBDR assay (line-probe assay) will be used for rapid detection of MDR-TB at baseline (according to the directions of the manufacturer). Indirect DST methods will be used to confirm results of the HAIN test and evaluate resistance to ethambutol and pyrazinamide. Testing will be performed in the BACTEC960 MGIT system with the SIRE kit (one each lyophilized vials of streptomycin, isoniazid, rifampin and ethambutol and eight vials of SIRE supplement and the PZA kit).

Using aseptic techniques and specified quantities, each drug will be reconstituted into stock solution. Inocula will be prepared from pure cultures of *M. tuberculosis* and suspended in Middlebrook 7H9 medium. The suspension will be vortexed and adjusted, ultimately, to ≥0.5 McFarland Standard for turbidity and then diluted (1:5). All MGIT DSTs will be performed with a growth control. Check for bacterial contamination will be performed at 48 hours; testing will be repeated if growth on blood agar is detected. The BACTEC 960 MGIT will monitor the tests until a result is determined.

A sample of 15% of pretreatment cultures will be sent to SGUL for quality control of DST. Samples will be stored at the SES study lab until a sufficient number has accumulated to fill a biohazard shipping box. Shipments will be sent with cold bags at -70°C to SGUL until 15% (27 cultures) have been evaluated. If quality problems are detected, improvements will be implemented and the process will be repeated.

At SGUL, DSTs in liquid medium will be set up in the BactAlert system using a single bottle for each drug known in this system to be the optimal break point and growth will be monitored in this bottle as an indication of resistance. This program will require bottles containing the critical concentration for isoniazid, rifampin and ethambutol together with a drug-free control bottle.

DSTs in Middlebrook 7H11 will also be set up on each culture using the standard British Medical Research Council method. In addition pyrazinamide susceptibility will be tested by the Brander method at pH 7.0 on slopes containing 500, 1000 and 2000 μg/ml nicotinamide.[100] Additional tests for pyrazinamide will be carried out on acid LJ medium. Amidase activity will be tested with an ammonium ion electrode.

8.3 Special Assays or Procedures

8.3.1 PK Sampling

After no fewer than 13 days of RIF and companion drugs administered by DOT, patients will be admitted to one of the study research centers. The preference is to draw the PK blood sample at the 5th overnight sputum sample collection in order minimize the number of patient visits. PK sampling may be scheduled, however, on any single day between treatment day 14 and day 56, with > 3 prior doses administered on consecutive days prior to sampling.

At the study research center, participants will be interviewed to obtain additional information about recent weight loss or weight gain, concomitant medications on the day prior to and the days of PK sampling, and gastrointestinal symptoms. Each participant will be weighed and their vital signs recorded. Urinalysis will be performed (proteinuria) and sputum samples will be collected for the LipTox Red substudy.

Participants will be observed to abstain from food for four hours prior to and for two hours after drug administration on the day of PK sampling. Water will be allowed, as desired. A small bore intravenous cannula will be inserted aseptically into a forearm vein, secured and its patency maintained using heparin or saline flushes. Baseline blood samples will be collected for PK (10 mL total). Scheduled study medication will then be administered according to the participants' assigned dose.

120 participants will be randomly selected during the pre-dosing visit for sparse PK sampling. They will have two further PK samples, 10 mL at each time point, drawn at 2 and 6 hours after drug administration.

60 participants will be randomly selected for participation in the intensive PK study. They will have 6 further samples, 10 mL at each time point, according to an optimized sampling scheme determined from existing and pilot data in Peruvian tuberculosis patients. Their baseline sample will be followed by samples collected during six sampling windows (15-45 mins, 45-75 mins, 75-105 mins, 105-135 mins 345-375 mins and 825-855 mins after dosing).

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

8.3.2 Pharmacogenetic testing

One baseline blood sample will be collected for pharmacogenetic analyses. These specimens will be tested for polymorphisms in SLCO1B1 and NAT2, which have been linked to variability in absorption. If different rates of hepatotoxicity are observed among the treatment arms, specimens will be analyzed for polymorphisms in NAT1, CYP2E1, and GSTM1, which have been linked to hepatotoxicity in patients receiving anti-TB therapy.

8.3.3 Genotyping

Baseline *M. tuberculosis* isolates will be stored frozen at -70°C, labeled with the study identifier number and the date of sputum collection. Stored *M. tuberculosis* isolates will be tested by genotyping methods (MIRU-VNTR) in case of recurrent disease among a study participant during the follow-up period.

8.4 Specimen Preparation, Handling, and Shipping

We will follow standard procedures for the preparation, handling, and shipping of blood (for PK analysis), raw sputum, and frozen sputum and cultures.

Procedures are summarized here.

8.4.1 Instructions for Specimen Preparation, Handling and Storage

8.4.1.1 *Bacteriology*

Quality of sputum, as a diagnostic specimen, is dependent on the composition (saliva vs. sputum), degree of contamination, and cold chain maintenance. Failure to maintain a cold chain will result in a lower culture positivity rate and increased contamination rate. Quality of samples also degrades over time so all sputum samples will be used within 72 hours (3 days) of collection. Efforts will be in place to ensure cold chain maintenance and biosafety with handling of sputum cups, since mycobacteria are often found on the outside of sputum cups.

Procedures:

1. After specimen collection, labeling and recording, the specimen will be stored in a refrigerator (between 2° and 8°C) as soon as possible.
2. Measures for quality control will be implemented.
3. Specimens will be transported in a closed container with ice packs, to maintain the cold chain, and transport temperature should be quality controlled.
4. Specimen tracking forms will accompany specimens, but will be stored separately (to prevent contamination). They will be used to assure complete transfer of specimens.
5. Sputum specimens will be delivered on working days to the microbiology lab, before midday when possible. All specimens will be processed, stained, and read as soon as possible, preferably within 24 hours of collection.
6. Cultures (LJ, MGIT, & Middlebrook) and DST will be inoculated as soon as possible, preferably within 48 hours of sputum specimen collection.
7. Isolation of DNA for line-probe assay (HAIN test) will be achieved as soon as possible, preferably within 24 hours of collection.
8. The 15% of pre-treatment cultures to be sent for QA of DST will be frozen at -70C in cryovials labeled with patient and specimen IDs in the freezer in the microbiology lab.

The microbiology samples that will be stored for potential later or long-term use are: 1) isolated DNA from 1 pre-treatment culture used both for molecular typing in case of recurrent disease and for the complex infection substudy, and 2) isolated DNA from, and all cultures performed on, 2 on-treatment pooled sputum samples for the complex infection substudy. Study participants will be asked to provide informed consent specific to storage and use of each of these specimens.

8.4.1.2 *Pharmacokinetics and Pharmacogenomics*

Procedures:

1. All PK samples are venous blood drawn into a heparinized collection tubes.
2. PK samples, after being drawn into 10 ml heparinized tubes and centrifuged, will be placed in ice slurry (packed ice & water).
3. Plasma for each patient at each sampling time point will be pipetted into cryovials and frozen at -20°C as soon as possible (preferably within 1 hour of the blood draw so that degradation of the drug does not occur). The cryovials will then be transported to the SES laboratory where they will be stored at a temperature of -70°C.
4. Labeled plastic EDTA tubes with 2 mL blood sample for pharmacogenetic analysis will be stored (unprocessed) in the -70°C freezer with the plasma samples.
5. Times of specimen collection, processing, and freezing will all be recorded.
6. Freezers temperature will be monitored and documented.

8.4.2 Specimen Shipment

Susceptibility testing, SSCC, and other solid and liquid culture bacteriology will be performed at the SES microbiology lab; local transport will be effected according to the procedures outlined above.

For international shipment of specimens, HIRIF will contract with an experienced shipper of biohazardous materials. A small number of cultures and sputum specimens will be sent to St George's, University of London; frozen sputum will be sent to Liverpool for the LipTox Red substudy. We will have export permits for shipping sputum specimens out of Peru and import permits for receiving them in the UK. These specimens are considered biological materials, which require a permit for importation. Shipments will contain proper documentation (printed on official letterhead) for the appropriate authorities in the UK. Specifically, St. George's will do the following.

1. A sequential sample of 15% of the patients will have a pretreatment culture tested by a standard DST method as a check on the DST methodology. Packages containing not more than 60 ml of infective material may be sent as accumulated. They will be sent packed in dry ice to maintain temperature, via an experienced shipper of biohazardous materials.
2. Quality control of CFU counting will be performed on a small (50), non-random sample of overnight, pooled sputum specimens at SGUL. Samples containing at least 3 ml of sputum will be eligible for use in this quality control. One ml of sputum will be separated and sent with cold bags to maintain temperatures between 2 and 8 degrees Celsius, via a commercial, authorized biohazard carrier, at four different times during the study. Specimens to be contained in these shipments may be refrigerated in a temperature-controlled refrigerator at the microbiology lab for up to 1 week prior to shipment. There will be no more than 60 ml of infective material per shipment and no more than four shipments. These will be set up by the standard method for comparison with the results from the SES microbiology lab. Timing of shipping of PK specimens

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

will be arranged with the Lima site, based on pace of enrollment and sample volumes. Samples will remain frozen at -70C until assayed.

Divided PK samples (no two samples from a single patient at a single time point will be sent in the same package) will be batch shipped to Dr. Charles Peloquin (Infectious Disease Pharmacokinetics Laboratory, College of Pharmacy, and Emerging Pathogens Institute University of Florida, 1600 SW Archer Rd., Rm P4-33, Gainesville, FL 32610-0486) in compliance with relevant Federal and International regulations. Shipping labels with the universal biohazard symbol, the address of the importer (Dr. Peloquin), the permit number, and the expiration date, are also issued to the importer with the permit. The importer must send the labels and one or more copies of the permit to the HIRIF site investigators. The permit and labels inform the U.S. Customs Service and U.S. Division of Quarantine Personnel of the package contents

8.4.3 PK Assays: Rifampin, Isoniazid, Pyrazinamide, and Ethambutol

Validated techniques that have been inspected and approved by the College of American Pathologists (CAP) will be used to determine the plasma drug concentrations of the 4 drugs, as previously described in publications by Dr. Peloquin.[101-103] In prior studies, the absolute recovery of rifampin from serum was 95.5%. The within-day precision (%CV) of validation quality control samples was 2.4 to 4.6%, and the overall validation precision was 6.3 to 7.1%. Comparable specifications are recorded for the other 3 drugs in the validation reports. The standard curves for the rifampin concentration in plasma covered a range from 0.5 to 50 ug/ml.

Because these assays will be identical to those used in previous clinical studies, the results should be directly comparable. Rifampin and isoniazid will be assayed using high performance liquid chromatography (HPLC); pyrazinamide and ethambutol will be measured using gas chromatography/mas spectrometry (GC/MS).

The specimens stored for the complex infection sub study will not be transferred out of Peru under this protocol.

9.0 ASSESSMENT OF SAFETY

9.1 Specification of Safety Endpoints

Safety endpoints are listed in Section 4.3.4.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

ICH E6 defines an adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor. The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment.

All AEs must be graded for relationship to study product.

9.2.1.1 *Assessing AEs*

Study participants will be monitored and assessed clinically for AEs at study visits at weeks 1, 2, 4, 6, 8 according to procedures specified in section 7. Daily visits for supervised treatment will provide additional opportunities for symptomatic screening and referral for unscheduled visits for potential AEs. Lab screening for hematologic and biochemical abnormalities will be conducted at study visits at weeks 2, 4, (6 for biochemistry only) and 8. Clinical visits during the continuation and follow-up phase will also assess delayed AEs at weeks 12, 16, 20, 24, 36, and 48. AEs of grade two or higher during the intensive phase, and up to four weeks after, are toxicity endpoints in this study.

9.2.1.2 *Severity of Event*

All clinical and laboratory findings (solicited and unsolicited) will be graded by clinical investigators according to severity using the modified DMID Adult Toxicity Tables (see *Figure 8* in section 17.6). Please see Table 9 for modified study-related toxicity criteria. The following serves as a general guideline to quantify intensity.

- **Grade 1 Mild** asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Events require minimal or no treatment and do not interfere with the patient's daily activities.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

- **Grade 2 Moderate** minimal, local or noninvasive intervention indicated; limits age-appropriate instrumental ADL.[4] Events result in a low level of inconvenience or concern with the therapeutic measures. May cause some interference with functioning.

- **Grade 3 Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limits self-care ADL.[5] Events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

- **Grade 4 Life-threatening** consequences; urgent intervention indicated. Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred. Events that are considered life-threatening meet SAE criteria and will be reported to the sponsor by the site within 24 hours of awareness of the event.

- **Grade 5 Death** related to AE. Death is an event outcome that meets SAE criteria and will be reported to the sponsor by the site within 24 hours of awareness of the event.

9.2.1.3 *Relationship to Study Regimen*

All AEs must have their relationship to study product assessed Peruvian standards, relationship will be assigned by local investigators using the following terms: not related, definitely related, probably related, possibly related, and probably not related. For the purpose of reporting to DMID, tracking events towards the halting rules, or any other safety oversight activities, only the terms related or not related will be used. They are defined as follows:

- Related – The event is temporally related to the administration of the study product and no other etiology explains the event. This will include all events assessed by investigators to be definitely related, probably related, possibly related, or probably not related.

- Not Related – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

9.2.2 Serious Adverse Events

A serious adverse event is defined as an AE meeting one of the following conditions:

- Death during the period of protocol defined surveillance
- Life threatening event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

[4]Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[5] Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All serious adverse events will be:

- Recorded in a timely and proper manner, and reported to the relevant entities.
- Followed through resolution by a study clinician
- Reviewed and evaluated by a study clinician

9.2.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

In this section, lab values for grading of AEs/SAEs are specified. Management procedures are also detailed, including continuation or suspension of study medication, additional evaluations, and clinical management of adverse events.

Grading according to lab values

All adverse events will be graded, and their severity and relationship to study drugs will be established according to criteria established in section 9.2.1. Laboratory values will be used for grading as specified in Table 9. Values in Table 9 have been derived mostly from the DMID Adult Toxicity Table (November 2007 draft), completed with criteria from the NCI CTCAE version 4.0, and modified to reflect normal lab values in the clinical lab in Peru. This includes only values for laboratory tests carried out routinely during HIRIF:

- Hematology: CBC with differential, platelet count
- Biochemistry: AST/ALT/ALP, bilirubin, albumin

Any other unexpected AEs or SAEs will be graded according to the DMID Adult Toxicity Table (November 2007 draft, *Figure **8***).

Management

In general, for all toxicities that are treatment-emergent and that require the study therapy to be temporarily or permanently discontinued, relevant clinical and laboratory tests will be obtained as clinically indicated and repeated as needed until final resolution or stabilization of the toxicity.

For **grade 1 toxicities**, the participant will be followed more frequently by clinical study staff than specified in the schedule of visits; study drugs will be continued.

For **grade 2 toxicities**, the participant will be followed more frequently, with additional laboratory and clinic visits as necessary. The study drugs may be continued or temporarily held at the investigator's and/or independent safety monitor's discretion. For Grade 2 thrombocytopenia (platelet count <75,000/cu mm) attributed to RIF, RIF should generally not be restarted.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

For any **grade 3 toxicity,** which, in the clinical site PI's judgment is NOT related to study drug(s), the participant will be followed more frequently, with additional laboratory and/or clinic visits as necessary. When possible, concomitant medications should be held first, at the discretion of the investigator, if he/she suspects they are contributing to toxicity. The study drugs may be continued or temporarily held at the investigator's and/or independent safety monitor's discretion, or if they are suspected to contribute to toxicities due to concomitant medications.

For any **grade 3 toxicity,** which, in the clinical site PI's judgment is related to study drug(s), the causative study drug(s) should be held. At clinical site PI's discretion and guided by local standard practice, it would also be acceptable to continue treatment, in conjunction with additional clinical and laboratory monitoring, and suspend treatment if the participant's condition or test result worsens. The clinical site PI should exclude other possible causes of the event before suspending study drug(s). When possible, concomitant medications should be held first at the discretion of the investigator if he/she suspects they are contributing to toxicity. Depending on the nature and severity of the toxicity, the degree to which it resolves, and/or the emergence of alternative explanations for the toxicity or the subject's deterioration, the study drug(s) may be restarted at the discretion of the investigator. In the case of Grade 3 hepatoxicity, ALT>3 x ULN with symptoms, >5x ULN without symptoms, per the guidelines of the American Thoracic Society—the drug can be restarted only if the subject's ALT returns to baseline level, <2 x ULN.[49]

Any participant with **grade 4 toxicity** will have study therapy immediately suspended. The laboratory test or clinical finding in question will be reassessed as soon as possible. The repeat test will guide management of the event as follows:

- If the repeat test shows grade 4 toxicity, then the patient will be permanently discontinued from study medications. Further treatment of TB will be directed by the investigator on an individualized basis, according to local standard of care. The patient will continue to be followed in the study.
- If the repeat assessment shows toxicity of grade 3 or lower, and if the patient has received study drugs between the two testing dates, then the patient will be managed according to the toxicity level of the repeat test. If the repeat test shows toxicity of grade 3 or lower, and if the patient has not received study drugs between the two testing dates, then the re-introduction of study drugs is at the discretion of the investigator with input from the ISM, DMID and SMC.

For any recurring grade 3 or grade 4 toxicity the study drugs should be temporarily held and may be permanently stopped at the discretion of the investigator and/or the ISM, DMID and SMC.

If a patient develops hepatic toxicity requiring study drug discontinuation, the following evaluation will be undertaken: assessment for history of injection or non-injection drug use, alcohol ingestion, and use of other hepatotoxic drugs.

Table 9 *Toxicity Criteria-Routine Lab Testing and Temperature Measurement During HIRIF Study*

Adverse Event	**Grade**					
	1	**2**	**3**	**4**	**5**	**Source**
Hematology						
Hemoglobin	9.5-10.5 mg/dL	8.0-9.4 gm/dL	6.5-7.9 gm/dL	<6.5 gm/dL	-	DMID, 2007, draft
Platelets	75,000-99,999/mm^3	50,000-74,999/mm^3	20,000-49,999/mm^3	<20,000/mm^3	-	DMID, 2007, draft
WBC-high	>11,000-13,000/mm^3	>13,000-15,000/mm^3	>15,000-30,000/mm^3	>30,000/mm^3	-	DMID, 2007, draft
WBC-low	<LLN–3000/mm^3	<3000-2000/mm^3	<2000-1000/mm^3	<1000/mm^3	-	NCI, 2010, CTCAE version 4.0; adapted for Peru clinical lab
Biochemistry						
Creatinine	1.1-1.5x ULN	1.6-3.0x ULN	3.1-6x ULN	>6x ULN or dialysis required	-	DMID, 2007, draft
AST (SGOT)	<2.0x ULN	2.0- <3.0x ULN	3.0-8.0x ULN	>8x ULN	-	DMID, 2007, draft
ALT (SGPT)	<2.0x ULN	2.0- <3.0x ULN	3.0-8.0x ULN	>8x ULN	-	DMID, 2007, draft
ALP	<2.0x ULN	2.0- <3.0x ULN	3.0-8.0x ULN	>8x ULN	-	DMID, 2007, draft
Bilirubin (when accompanied by increase in other liver function test)	1.1- <1.25x ULN	1.25- <1.5x ULN	1.5- 1.75x ULN	>1.75x ULN	-	DMID, 2007, draft
Bilirubin (when other liver function tests are in normal range)	1.1- <1.5x ULN	1.5- <2.0x ULN	2.1 - 3.0x ULN	>3.0x ULN	-	DMID, 2007, draft
Albumin	<LLN-3 g/dL	<3-2 g/dL	<2 g/dL	--	-	NCI, 2010 CTC AE Version 4.0; adapted for Peru clinic lab
Systemic						
Fever, oral	37.7 – 38.5C or 100.0 – 101.5F	38.6 – 39.5C or 101.6 – 102.9F	39.6 – 40.5C or 103 – 105F	>40.5C or >105F	-	DMID, 2007, draft, overlap corrected

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

9.3 Reporting Procedures

This reporting system has been developed to ensure timely and accurate reporting of adverse events in order to monitor patient safety. It also assures compliance with Department of Health and Human Services (HHS) and Food and Drug Administration (FDA) regulations, in-country regulations, as well as dissemination of information to investigators working with the study drugs. In accordance with the FDA's Code of Federal Regulations, the sponsor of this clinical trial and the participating investigators are responsible for reviewing all information relevant to the safety of the study drugs. Reporting and monitoring of SAEs are required to alert the FDA, sponsor, institutional review boards, and the investigators of real and potential safety issues.

The study drugs are ethambutol, isoniazid, rifampin, and pyrazinamide. The most common adverse effects associated with the study drugs are specified in section 2.3. The investigator is responsible for monitoring all adverse events that are observed or reported during the study, regardless of whether they are related to study drugs.

9.3.1 Adverse Events

- Document AEs from the first study intervention, Study Day 1, through Study Day 365 (end of follow-up).

Adverse events including local and systemic reactions not meeting the criteria for "serious adverse events," should be captured on the appropriate case report form. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study product, time of resolution of the event, seriousness, and outcome. All adverse events occurring during the first three months of treatment must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study it should be recorded as an AE. The maximum level of toxicity reached will be clearly indicated.

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

9.3.2 Serious Adverse Events

- Document SAEs from the first study intervention, Study Day 1, through Study Day 365 (end of follow-up).

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.
Any AE that meets a protocol-defined serious criterion must be submitted within 24 hours of site awareness on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

Any AE that meets a protocol-defined serious criterion must be submitted within 24 hours of site awareness on an SAE form to the IND holder. If the IND holder deems regulatory reporting of the SAE necessary, an IND safety report will be submitted to the FDA. Submission will be realized within 15 calendar days of the date on which reporting was determined to be necessary.

9.4 Reporting of Pregnancy

For the purposes of this study, pregnancy will be reported as an adverse event. All pregnant patients will be taken off study therapy and treated according to local TB program guidelines for tuberculosis treatment during pregnancy. Women who become pregnant while on study treatment will, with their permission, receive follow up for 14 days after discharge from the study; newborn will be followed for 6 months. Pregnancy will be reported to the local IRB and Partners IRB as an unanticipated problem involving risks to subjects or others.

9.5 Type and Duration of Follow-up of Subjects after Adverse Events

Adverse event follow-up will be reported on a designated form as soon as possible after the resolution or stabilization of the AE, but no later than 45 days after the AE was reported. An SAE will be reported by the study site to the sponsor within 24 hours of awareness of the event. AEs and SAEs will be followed, until resolved or considered stable, per the study schedule of evaluations, or more often at the discretion of the investigator. The investigator will submit any information regarding follow-up on the SAE as soon as possible after the information becomes available to the study site. A copy of the completed AE report and any FDA-related communications to DMID will be reported within 7 days of becoming available.

9.5.1 Halting Rules

The Chairman of the SMC or his/her designee will review each SAE deemed to be related to rifampin within 48 hours of report. The Chair or his/her designee will determine whether the SAE requires halting of the trial pending consideration by the full SMC. In addition, the SMC will be notified if any of the following events occur and the trial will be halted pending SMC review and recommendations:

a) 3 or more SAEs of the same type, assessed by the PI, ISM, and MM to be related to rifampin.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

b) 6 or more grade 3 or higher AEs related to rifampin, excluding elevated biochemical transaminases.

c) 3 grade 3 or higher elevated biochemical transaminases accompanied by clinical symptoms consistent with hepatotoxicity, related to rifampin.

d) 1 or more systemic hypersensitivity reaction (anaphylaxis) within 24 hours after receiving rifampin and related to rifampin.

If, in accordance with the above halting rules, the study is temporarily halted and then restarted, the count of events that invoked the rule will restart at "0".

The SMC, PI, and DMID retain discretion to halt the study for other safety concerns (or based on interim safety results) until a safety evaluation is performed by the SMC, if warranted by frequency, nature, or severity of recorded events. The SMC will review all of the safety data and recommend whether enrollment and dosing should resume. Subsequent review of serious, unexpected, and related AEs by the SMC, IEC/IRB, the Sponsor, or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site.

The Sponsor will notify the FDA if the study is halted. A decision to reinitiate the study will be made based on the recommendation of the SMC and the sponsor.

In the event of a study safety halt, subjects will be treated with a standard regimen for TB according to local guidelines. When and if it is determined that the study may continue, participants in the intensive phase of treatment will reinitiate their assigned study regimen.

9.6 Contingency Plan for Unblinding

The site principal investigator will not have access to the randomization code indicating the investigational treatment arm assigned to a participant except in the case of an emergency and when knowledge of treatment arm assignment is essential to ensure the immediate safety and clinical management of the participant.

The site principal investigator is the only member of the study team who has the authority to unblind the study, and only in the interest of ensuring study participant safety. Afterwards, s/he will report the unblinding promptly (within no more than 72 hours after unblinding) to the study sponsor and the DMID Medical Monitor. The Safety Monitoring Committee may request unblinded data in order to ensure the safety of study participants.

The documentation regarding unblinding must be registered in the participant's clinical history and must be captured in the study data base, including the date and time in which the change took place, and the names of all the staff involved. Additionally, the AEs that lead to the unblinding must be recorded in the appropriate study CRF. Once a study participant's treatment assignment is unblinded, that participant will not be able to restart study treatment.

9.7 Safety Oversight

An Independent Safety Monitor (ISM) and a Safety Monitoring Committee (SMC) will provide safety oversight.

The ISM will be a physician with expertise in TB treatment and clinical research. The ISM will not be under the direct supervision of the PI, nor will s/he be considered a member of the SMC. He/she will be responsible for providing independent safety monitoring in a timely fashion. Participation is for the duration of the study. The ISM will review any SAEs and unexpected AEs of concern, immediately after they occur, and follow-up through resolution. The ISM will have ready access to participant records and will be provided a copy of the SAE at the time the SAE report is submitted to the DMID pharmacovigilance group. He/she will independently review all SAEs and thoroughly investigate those events considered unexpected. Clinical and laboratory data, clinical records, and other study-related records will be made available for ISM review, by local investigators and/or coordinators. The clinical PI will ensure that the ISM is apprised of all new safety information relevant to RIF and companion drugs.

The ISM will review all protocol revisions and may receive other documents related to the study. The ISM may transiently or permanently halt enrollment at any time for any safety related issue. The ISM will contact the PI at the enrollment site and the medical monitor at the DMID for any event that needs further evaluation. The ISM will provide a written assessment about all SAEs to the DMID. The DMID will communicate with the SMC chair.

The SMC will comprise one clinical TB expert, one internationally recognized clinical trialist, and one clinical researcher with expertise in a topic of relevance to the study (e.g., hepatotoxicity), none of whom will be otherwise involved in the study. A statistician may also participate, either as a full voting member or as a consultant to the SMC at open session meetings. S/he will not otherwise be involved in the study. Additional expertise may be added for open or closed meetings, as needed, on an ad hoc basis. A simple majority will be considered a SMC quorum. The SMC will meet after approximately three months, or after 1/3 of patients are enrolled to assess safety for each arm of the study. See sections 4.3.5 and 4.3.6 of protocol for details regarding SMC review and data to be reviewed for the interim analysis plan. If halting rules are invoked, more frequent meetings may be held. The SMC will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. The SMC will assess interim/cumulative data for evidence of study-related adverse events, data quality, completeness and timeliness, demographic information on study participants, factors that might affect the study outcome or compromise the confidentiality of the trials (such as protocol deviations) and factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study. The ISM or SMC may temporarily or permanently halt enrollment at any time for any safety related issue. The SMC should conclude each review with its recommendations to the study sponsor as to whether the study should continue without change, be modified, or terminated.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

10.0 CLINICAL MONITORING

10.1 Site Monitoring Plan

Under the U01 mechanism, site monitoring is the responsibility of the investigators. To ensure that human subject protection, study procedures, bacteriology procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs, the study will contract with an external monitor who will comply with the requirements laid out in the ICH GCP Guidelines E6, Section 5.18. This is detailed in the Clinical Monitoring Plan.

Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions in a formal report as specified in section 5.18.6 of ICH GCP Guidelines E6.

11.0 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

Primary:

H1: The distribution of AUC_{0-24} of RIF will be different among the three dosing groups.

H2: Sputum culture sterilization will occur more quickly in the intervention groups than in the control group.

H3: Adverse event (grade 2 and higher) incidence will be independent of dose size, AUC and C_{max} of RIF.

Secondary:

H4: Sputum culture sterilization will occur more quickly in study participants with higher RIF AUC/MIC.

H5: A relationship among four different methods of measuring sterilizing activity can be characterized.

H6: Participants with accelerated sputum culture sterilization will have a lower probability of failure and relapse.

11.2 Sample Size Considerations

11.2.1 Pharmacokinetics

Existing unpublished data suggest a minimum increase in AUC of 12 mcg/mL*hr across dose groups. Assuming a standard deviation of AUC of 24 mcg/mL*hr and α=0.05, a linear contrast test across the three treatment groups has 90% power to detect a total effect size of 14 mcg/mL*hr between the top and bottom dose levels at a sample size of 50 evaluable subjects per arm. We propose to recruit 60 subjects per arm which will allow up to 17% loss to follow up. With 50 evaluable patients/arm, the study has 90% power to detect a total effect size as small as 12.8.

11.2.2 Efficacy

Estimation of the sample size for the serial sputum colony counting endpoint is based on computation of the population Fisher's Information matrix derived from linearization of a non-linear mixed effects model for the data. The parameter θ_4 in this model represents the late phase decay in colony counts, a surrogate measure of sterilizing activity. Under conventional assumptions of α=0.05 and ß=0.20 with a coefficient of variation on this parameter of 20% a sample size of 48 per arm is sufficient to detect a difference between the highest and lowest dose arms of 0.025 $\log_{10}$ CFU/ml, comparable in magnitude to those observed in previous SSCC studies. A sample size of 60 per arm allows for a rate of patient withdrawals of up to 25%.

11.2.3 Safety

Hepatotoxicity has been observed to occur in between 0 and 27% of patients receiving RIF-containing regimens for TB, with the summary frequency from one meta-analysis estimated at 2.7%. Other serious toxicities, such as hematologic disorders and flu-like syndrome are estimated to occur in between 1 and

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

5% of patients on standard doses of RIF. In a pivotal trial of rifapentine (compared to standard doses of RIF) 5% of subjects receiving standard doses of RIF permanently discontinued treatment.[104] We expect 5% of patients in the control arm to experience adverse events requiring treatment discontinuation; we expect at least twice this many, or 10%, to experience a grade 2 or higher event.

We have 62% power (1-sided α=0.1) to detect adverse events occurring twice as frequently in the intervention arms combined, and greater than 95% power to detect a relative risk of $\geq$3. We have greater than 70% power to rule out that there is a tripling of risk in one of the intervention arms, compared to the control arm. If we assume the frequency of any adverse event (greater than grade 2) is higher, 10% in the control arm, we have 68% power (1-sided α=0.1) to detect adverse events occurring twice as frequently in the intervention arms. Since we will be comparing incidence of adverse events, a continuous variable, the statistical power afforded by the study sample is size is actually slightly higher.

11.3 Final Analysis Plan

More details will be available in a full statistical analysis plan completed prior to initiation of any analyses.

All initial analyses will be intent to treat. All analyses will examine the effect on specified endpoints of increased doses of RIF. Distribution of potentially important covariates among treatment arms will be assessed. These include: age, gender, cavitation, HIV, RIF MICs, and baseline bacillary load. Any imbalance will result in adjustment in multivariable analyses for covariates that are not evenly distributed among the treatment arms. Study participants who are lost to follow up will contribute information until their loss to follow up. Informative missingness will be assessed for any missing endpoints and covariates.[105] Results of these assessments will inform handling of missing data in subsequent analyses.[106] Plans include full case analysis and imputation for PK, efficacy, and safety; and integration of separate likelihoods (PK). An analogous approach will be applied to informative censoring in the time-to-event models. General approaches are outlined below.

11.3.1 Pharmacokinetic-Pharmacodynamic analysis

These analyses will address the primary question of whether AUC_{0-24} varies by treatment arm.

11.3.1.1 *Data cleaning, transformation and missing data*

Plasma RIF concentrations (μg/mL) divided by RIF MIC will be used as the primary endpoint. Missing concentration values for protocol or technical reasons will be assigned NA. Values below the Limit of Detection (LOD) of the assay will be handled by creating two datasets: one in which these values are also assigned NA and another in which a value half the LOD is assigned. Analysis with the imputed values will serve as an assessment of the degree to which these LOD observations may be informative. The dataset will also be prepared in NONMEM format and values below the limit of detection coded as MDV=1.

We anticipate that parametric methods will be appropriate since, based on prior reports, we do not expect the distribution of RIF plasma concentrations to violate the normality assumption. We will, however, evaluate this assumption and adopt non-parametric analytic approaches if the distribution of RIF plasma concentrations is non-normal.[107]

11.3.1.2 *Data summaries and descriptive statistics*

Plasma RIF concentrations will be plotted raw and grouped by subject on the original scale to detect outliers. MICs will also be plotted. Summary statistics (mean, median, quartiles and standard deviation) will be computed for PK at each individual time point and for RIF MICs to evaluate the distribution of the data. Outliers will be retained in the dataset but any extreme cases may be queried with the laboratory, re-analysed and potentially removed by consent of the study team. Standard summary pharmacokinetic parameters (C_{max}, t_{max}, AUC_{0-24}, AUC_{0-6}, $AUC_{0-\infty}$ and total clearance) will be obtained using non-compartmental analysis and summarised graphically and numerically as described above by dose group. Percent extrapolation for AUC_{0-24} will also be recorded for each subject. Ratios of AUC_{0-24}/MIC will also be calculated for each patient.

11.3.1.3 *Prespecified analyses*

The primary analysis will be a two-sided linear contrast test of dose-response of AUC_{0-24}/MIC across the three dose groups with a significance level of 5%.

Secondary analyses of the summary parameters will include similar tests of dose-response for $AUC_{o-\infty}$ and C_{max}. If departure from linearity is evident from plots of the PK parameters, alternative contrasts (concave or convex) or polynomial terms will also be fitted to obtain better estimates of the dose-response relationship. Exploratory analyses of additional determinants of the exposure parameters (AUC_{0-24}, $AUC_{o-\infty}$ and C_{max}) and of clearance will include the covariates study site, body mass, sex, and plasma concentration of companion drugs.

Population PK modelling of the parent compound and its major metabolite, 25-desacetylrifampicin, using the rich and the complete datasets will also be carried out. A one compartment model for the parent compound alone and a simultaneous parent-metabolite model will be fitted. Similar dose-response contrasts and covariates will also be examined in this model.

We will use data from the small number of MICs of 25-desacetylrifampicin for exploratory analyses of the possibility that the metabolite adds to RIF's activity.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

11.3.1.4 *Statistical methods*

Non-compartmental analysis (NCA) will be carried out using *WinNonLin* 5.0 (Pharsight Co, USA). T_{last} will be selected automatically or confirmed by the analyst for profiles with more than two data points. The trapezoidal rule with the log up-linear down option will be used to fit the profiles. The summary PK parameters will be analysed using analysis of variance in (SAS version 9.1, The SAS Institute, Cary, NC).

Population PK modelling will be carried out in NONMEM VI (ICON) via R and the packages NMFuns and Mifuns (Metrum Institute). The Laplacian and/or First Order Conditional Estimation method with ε-η interaction will be used. Covariate selection will be done using graphical screening methods, univariate fitting of candidates with a conservative level of α=0.10 and backwards elimination from the final model with α =0.05. Model comparison will be based on changes in the NONMEM objective function/Likelihood ratio test, examination of weighted and conditionally weighted residual plots and posterior predictive checks. Missing observations will be handled in three ways: first by ignoring the missing observations, secondly by imputing a value of half the LOD and thirdly by separately integrating the likelihood for these missing observations using a purpose written subroutine. Covariate selection will be done using graphical screening methods, univariate fitting of candidates with a conservative level of α=0.10 and backwards elimination from the final model with α=0.05.

11.3.2 Efficacy analyses

These analyses will answer the secondary question of whether observed sterilizing activity, particularly in the latter part of the intensive phase, varies by treatment arm.

11.3.2.1 *Data cleaning, transformation and missing data*

Replicate sputum colony counts will be averaged and transformed to the $\log_{10}$ scale. Since the counts are performed on cultures grown from homogenized sputum samples, they will not be normalized by sputum volume; previous work reveals that such normalization does not decrease variability in colony counts in most populations.[108] Counts not available will be distinguished as to the reason for being missing: not collected vs. no sputum produced vs. contaminated vs. below LOD. The first three will be coded NA. The point of culture conversion will be defined as the first value below the LOD in an individual subject's profile ending in at least two such values. Two distinct datasets will then be created: one in which the below LOD observations are all coded NA and a second which the value at the point of culture conversion is imputed a value of half the LOD and all successive values coded NA.

Liquid culture results will be recorded as DTP and all bottles will be declared negative at 42 days of incubation. For the survival analysis dataset this time point will represent culture conversion, the event of interest, and all profiles not achieving this endpoint will be censored at last SSCC/DTP sputum collection time point. For the regression analysis of the DTP data, DTP will ideally be analysed on the original scale but may be transformed to stabilize the variance. A similar approach to missing data will be adopted as above but with a value of 42 days imputed at the point of culture conversion.

11.3.2.2 *Data summaries and descriptive statistics*

Both SSCC and DTP data will be plotted and basic descriptive statistics computed at all time-points in order to assess heteroscedasticity and detection of outliers. Kaplan-Meier plots will also be created according to dose level.

11.3.2.3 *Prespecified analyses*

The co-primary efficacy comparisons will be based on a two sided linear contrast test of the three dose levels on the parameter θ_4 (late-phase sterilizing slope) derived from non-linear mixed effects modelling and the hazard ratio of culture conversion derived from the Cox proportional hazards model at a significance level of 5%.

11.3.2.4 *Statistical methods*

SSCC data will be analyzed using a non-linear mixed effects (NLME) model employing maximum likelihood estimation in the package NLME in R. The basic structural model will be a four parameter biexponential decline with random effects for all the parameters and a variance function to account for heteroscedasticity. The following covariates will be examined for inclusion in the adjusted model: HIV status, sex, age and extent of disease (radiography), RIF AUC/MIC. Model comparison will be based on the Likelihood Ratio Test for models estimated by maximum likelihood and on examination of residual plots. The final model will also be checked for residual serial correlation and stability of variance components under REML estimation. The analysis will be repeated for the imputed dataset to check robustness of the conclusions of the analysis to missing data.

We will assess whether the assumption of proportionality necessary for Cox proportional hazards models is met, examining plots of the natural logarithm of the negative natural logarithm of the survival curve. If so, Cox proportional hazards regression will be carried out using the survival package in R. The exact partial likelihood method will be used to resolve ties and comparison of models will be on the basis of the likelihood ratio test and examination of martingale residuals. If not, a non-parametric survival model will be used.

DTP data will also be analysed using NLME in R. Linear, monoexponential and spline structural models will be tested with a variance function if necessary. The same set of covariates will also be examined and incorporated according to the same selection criteria.

11.3.3 Toxicity

To evaluate the effect of dose size on adverse events (grade 2 or higher), the primary analysis will be a comparison of time to adverse events occurring in the first 12 weeks of treatment and determined to be related to the study drug or regimen in the intervention groups to the time to adverse events in the control group.

Secondary analyses will examine differences in time to all SAEs—and specifically in time to hepatotoxicity—requiring treatment discontinuation.

11.3.3.1 *Data cleaning, transformation and missing data*

The time to an adverse event will be recorded as the interval between the treatment initiation date and the midpoint between the last study visit without an event and the first study visit (scheduled or not) at which the AE was diagnosed. For participants lost to follow up after at least one study visit post treatment initiation, data will be censored at the midpoint between the last recorded study visit and the date of loss to follow up. Data from participants for whom no study visits occurred will be excluded from this analysis.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

11.3.3.2 *Data summaries and descriptive statistics*

Incidence rates of predefined and unexpected AEs and SAEs will be calculated. Kaplan-Meier plots will also be created according to dose level and stratified by severity grade (1-4).

11.3.3.3 *Prespecified analyses*

The incidence rate of grade 2 AEs, determined to be related to the study drug or regimen and occurring in the first 12 weeks, in the 2 intervention groups combined will be compared to the control group. Further the rate of discontinuation due to hepatotoxicity will be compared between the intervention and control groups. If a significant difference is detected, comparison will be made between the 15 mg/kg intervention group and the control as well as between the 20 mg/kg intervention group and the control.

RIF AUC and other covariates including age, sex, reported alcohol consumption and baseline LFT will be considered as covariates in multivariable regression of time to event, time to discontinuation (due to all SAEs), and time to discontinuation due to hepatotoxicity.

11.3.3.4 *Statistical methods*

The proportionality assumption will be assessed as described above. If met, Cox proportional hazards models will also be run to assess differences in time to AE between the intervention groups and the control group. If not, a non-parametric survival model will be used.

12.0 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

12.1 Source Documents and Access to Source Data/Documents

Appropriate medical and research records will be maintained for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

The following individuals and groups will have access to study records:

- Members of the study team
- IRBs that review the study (including IRB members, staff, and legal counsel)
- Office of Human Research Protections
- FDA
- DMID/NIAID/NIH
- Regulatory Agencies in Peru
- Study Monitor

Authorized representatives of the sponsor(s), DMID, and regulatory agencies indicated above will be permitted to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

13.0 QUALITY CONTROL AND QUALITY ASSURANCE

Standard operating procedures for quality management will be developed and detailed in a separate Quality Management Plan.

The executing institution is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Clinical site monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The Data Centers at each site (DC) will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the clinical site(s) for clarification and resolution.

All key study staff will be trained and certified in good clinical practices.

14.0 ETHICS/PROTECTION OF HUMAN SUBJECTS

The protection of human research subjects will be assured throughout the study execution and reporting.

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997). In addition, the investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject. It will also be conducted in full compliance with 21 CFR 50 and 56. In addition, this study will be conducted in accordance with Peruvian standards.

14.2 Institutional Review Board

Before they are placed into use, the study protocol and informed consent documents will be reviewed and approved by IRBs supervising all involved institutions (Harvard, St. Georges, University of Liverpool, University of Florida, Peru). Any amendments to the protocol or consent materials will be reviewed and approved by the IRBs before they are placed into use.

14.3 Informed Consent Process

Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided by study staff to potential subjects (and, with permission from potential subjects, their families). Potential subjects will receive counseling about study objectives and procedures, potential toxicities, and the informed consent process. Consent forms describing in detail the study interventions & products, study procedures and risks will be given to the subject and written documentation of informed consent will be required prior to starting intervention/administering study product. Consent forms will be IRB approved, will contain all the elements required by the ICH E6 Guidelines for Good Clinical Practice and any additional elements required by local regulations, and written in Spanish; English versions will be available for IRB and sponsor review. The subject will be asked to read and review the document. Upon reviewing the document, the person obtaining consent will explain the research study to the subject and answer any questions that may arise. For subjects who speak and understand the language used in the consent document, but are unable to read or write, all of the information in the consent form will be communicated verbally, in the presence of an adult witness who is not a member of the study team; informed consent requires the signature or mark of the subject. The subject will sign or mark the informed consent document prior to any procedures being done specifically for the study. The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the signed and dated informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing, to subjects, that the quality of their medical care will not be adversely affected if they decline to participate in this study. The informed consent process will be recorded in the subject's clinical chart.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

During the informed consent process, the subject will receive information about compensation for study participation. Specifically, subjects will not be paid for study participation. Study subjects will be reimbursed for transportation expenses to the research center they are assigned to and monthly food vouchers for the duration of time they are in the study. They will also receive meals while in the research center for PK and overnight pooled sputum collection. Study drugs will be provided free of charge.

14.4 Informed Consent/Assent Process (in Case of a Minor)

Not applicable. All study subjects will be ≥ 18 years of age.

14.5 Exclusion of Women, Minorities, and Children (Special Populations)

Children will be excluded from this Phase II study because, unlike in adults, there is limited information on the safety and tolerability of high-dose RIF among children. Pregnant and breast-feeding women will be excluded from this study because of the lack of evidence on the safety and tolerability of high-dose RIF among infants and fetuses. If its safety and tolerability among adults is confirmed with this study, research examining safety and tolerability among children will be planned.

14.6 Subject Confidentiality

This study will be performed in accordance with U.S., and Peruvian standards for protection of privacy of identifiable health information.

All study records will be managed in a secure and confidential fashion. Study records will be maintained in locked cabinets, and computer records will be password protected. Access to study records will be restricted to specified team members. Methods for secure data handling are detailed below in Section 15.0.

The study monitor, other authorized representatives of the sponsor, and U.S. and/or local regulatory authorities may inspect all documents and records required to be maintained by the investigators, including but not limited to, case report forms and pharmacy records for the subjects in this study. The clinical study sites will permit access to such records.

14.7 Study Discontinuation

In the event that the study is discontinued (e.g. by the investigator, the sponsor, the SMC, and/or regulatory groups), subjects on study phase treatment will continue to receive TB treatment in accordance with national guidelines. Treatment will be offered free of charge, as guaranteed through the public health system in Peru.

14.8 Future Use of Stored Specimens

Pharmacogenetic samples will be stored for up to 5 years. This is to permit testing for newly identified polymorphisms related to TB drug metabolism. Cultured organisms and mycobacterial DNA, which will be isolated from patient specimens at baseline and twice during the intensive phase, will be stored for analysis for complex infections.

Subjects will be asked to provide written informed consent allowing their blood samples and TB strains to be stored and analyzed for these studies. A section about the future use of specimens is included in the

consent forms. If a subject does not agree to consent to the future use of stored specimens, this does not preclude him or her from participating in the present study. Relevant institutional review boards will oversee any future research using these samples.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

15.0 DATA HANDLING AND RECORD KEEPING

The following types of forms will be used in this trial to ensure appropriate data handling and record keeping is performed:

Informed consent forms for enrollment, in English and translated into Spanish.

Eligibility screening forms

Medical records and case report forms that document enrollment, follow-up visits, adherence, and specimen collection

Administrative forms that document participant deaths, termination from study drugs, and work up and treatment of AEs and treatment failure

A participant's name will be collected only one time, and this information will be kept on a form that does not contain any test results, and that is filed separately from forms that do contain test results. Each participant will be assigned a unique study ID number. This number will be recorded on each data collection form and clinical specimen to facilitate linkage of data. The study ID number will be used on data collection forms; names and other obvious identifiers will not be used on data collection forms.

All data collection forms will be stored in locked files in a secure area. Access to study records and data files will be limited to study personnel, the NIH and its designees, the FDA, and local regulatory authorities.

All forms will be reviewed prior to data entry for accuracy, consistency, and completeness by designated study staff.

For banked specimens, the study ID number, date of collection, specimen volume, and freezer location will be recorded on the laboratory requisition forms and entered into the computer.

15.1 Data Management Responsibilities

The on-site principal investigator and the data manager will be responsible for the accuracy, completeness, and storage of source records and study data collection forms. The study team, monitor and data entry staff will review source documents and laboratory reports to ensure accuracy and completeness as appropriate. The site staff will maintain logs to record dates of completed and upcoming clinic visits and specimen collections.

15.2 Data Capture Methods

All information needed for the study will be entered into the electronic data capture (EDC) system and then verified.

15.3 Types of Data

Data for this study will include safety, laboratory (microbiology), and clinical data. All of these elements will be recorded in the EDC system.

15.4 Timing/Reports

Safety/tolerability data will be reviewed by the SMC after enrollment of each 60 (1/3 of) study subjects; reports for the SMC will be prepared for the SMC according to a schedule determined at the first convened SMC meeting. Data coding will occur at the time of data collection; ongoing logical data queries will be performed.

15.5 Study Records Retention

Within 2 years of completion of the study, identifiers excluding the study ID number will be deleted from computerized and paper data files. Study records will be maintained by the investigator for a minimum of 5 years following discontinuation of the study. The IND sponsor and DMID will approve study record destruction.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the sites and implemented promptly.

It is the responsibility of the sites to use continuous vigilance to identify and report deviations according to the guidelines of the IND sponsor. All deviations from the protocol must be addressed in study subject source documents. Protocol deviations must be sent to DMID and the local IRB in accordance with standard procedures.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

16.0 PUBLICATION POLICY

Following completion of the study, the investigator will plan to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This trial has been registered in ClinicalTrials.gov (NCT01408914). It is anticipated that the results of this study will be submitted for publication in a peer-reviewed scientific journal. The study PI(s) will retain primary responsibility for the preparation of publications. An NIAID Project Scientist will also have substantial involvement in providing scientific/programmatic support in the preparation of publications. Publications and oral presentations of work performed under this agreement will receive appropriate acknowledgment of support by the NIAID/NIH.

Authorship will be extended to the following individuals: study investigators, site PIs, and other individuals having major contribution to the study design, implementation, data analysis, and preparation of the written manuscript.

Prior to submission for presentation or publication, any materials derived wholly or in part from this study must be submitted to the study PI(s), site PI, and all co-authors for review.

17.0 PROTOCOL APPENDIX

17.1 Current Drug Development in Tuberculosis

The limitations of the current TB control strategy have resulted in renewed focus on development of effective "ultra-short-course" regimens. It has been estimated that successful introduction of an effective, inexpensive, two-month regimen could prevent 13 million infections and 6 million deaths in the 18 years following its introduction.[109]

Several such efforts, which could result in incremental progress towards this goal, are underway. The most advanced are Phase III trials designed to demonstrate the efficacy and regimen-shortening potential of the 8-methoxyfluoroquinolones moxifloxacin and gatifloxacin for first-line therapy. Preliminary results of the gatifloxacin-containing regimen do not support shortening with this regimen. Results from the study with the moxifloxacin-containing regimen are expected in 2014. Moreover, a number of chemically novel compounds are in pre-clinical testing or early clinical development, among them two nitroimidazoles (PA-824 and delamanid), a diarylquinoline (bedaquiline) and an ethambutol derivative (SQ-109). Clinical development of some of these compounds is at present focused on improving treatment of MDR-TB.[110] Phase III trials for first-line therapy with these compounds have recently begun. One of these drugs, bedaquiline, was conditionally approved for MDR-TB by the US FDA in December 2012 and by the EMA in 2013; delamanid also received conditional approval by the EMA in 2013.

The need for improved outcomes, however, is urgent: nearly 90 million new cases of TB and 20 million TB deaths can be expected over the next ten years. Moreover, the *Global Plan to Stop TB: 2006-2015* projected an estimated 11.7 million cases of TB/HIV coinfection and 3.4 million cases of multidrug-resistant TB (MDR-TB) by 2015.[111]

17.2 Measuring the Sterilizing Activity of Combination Regimens

Short-course regimens effectively reduce bacillary burden in the early phase of treatment, preventing death, bacteriological failure during treatment and the emergence of resistance. However, their inability to prevent relapse as the duration of therapy is reduced, despite favourable results during and at the end of treatment, led to the concept of "sterilizing activity". As noted, this has been defined theoretically by Mitchison as, "the ability to kill *all* or virtually all of the bacilli in the lesions as rapidly as possible"[4] in the context of the hypothesis of heterogeneous bacillary subpopulations *in vivo*. In particular, the phenomenon of "persistence" during therapy attributed to one or more subpopulations of metabolically inactive and antibiotic-tolerant organisms is now widely acknowledged to be a major limitation to shortening the length of current regimens.[112] In pre-clinical development this idea has led to the refinement of *in vitro* models differentiating drug activity between logarithmic and stationary growth phase bacilli and to improved animal models of chronic disease where sterilizing activity can be defined as the ability to render organs (lung, spleen) culture negative.

Recent advances using transcriptomics and imaging to examine clinical specimens have also confirmed the existence of the persister phenotype in clinical specimens.[113, 114] Quantitative studies of bacillary load in humans during short-course chemotherapy reveal that the rate of elimination of organisms slows dramatically as early as the second week of treatment with HREZ across populations (See *Figure 3* and *Figure 4*). Activity during this slow pharmacodynamics phase has been adopted as the operational definition of sterilizing activity by Jindani and colleagues.[115] Sterilizing activity is considered to be the key determinant of the length of the regimen: the greater the sterilizing activity, the shorter the regimen may be without substantial risk of relapse.[2] Since the relapse rate, the definitive measure of sterilizing

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

activity, is not a feasible endpoint for early clinical trials, a number of different bacteriological measures during the first two months of therapy have been proposed as surrogate endpoints. Recent re-evaluation of the historical series of short-course trials shows that such endpoints do have significant trial-level surrogacy for relapse. Across trials, however, a single bacteriological method, time point and form of analysis that best represent sterilizing activity has yet to be determined.[116] This is currently an area of research critical to the success of early clinical development efforts since boosting sterilizing activity is clearly the key to ultra-short treatment regimens for tuberculosis. However, providing reliable proof-of-concept for individual agents and effectively screening combination regimens for progress into Phase III trials can only be done with better surrogate endpoints for sterilizing activity.

Figure 3: Log10 CFU During Treatment with HRZE from 3 studies: Nairobi (red), Oflotub study (blue), Thailand (green) (Davies G, unpublished data)

Figure 4: Association Between the Treatment Effect on a Positive Culture and the Treatment Effect on a Poor Outcome (across studies comparing regimens containing RIF throughout)

Figure 3:

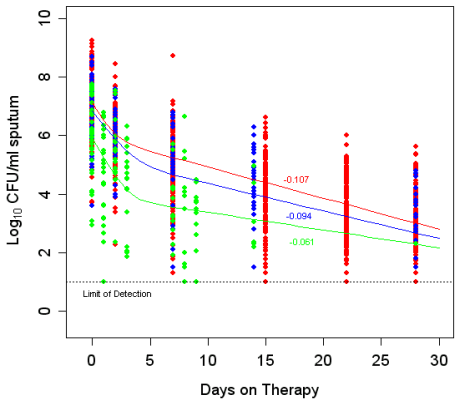
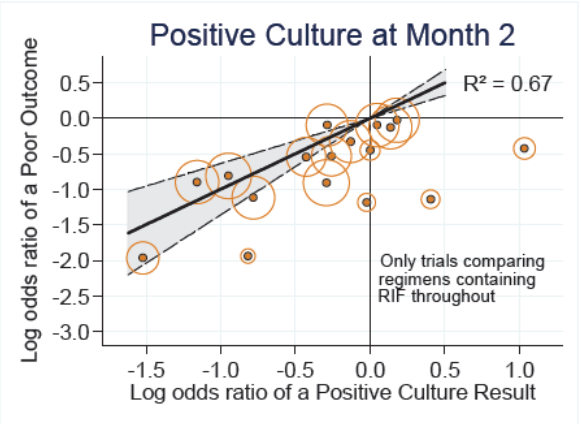


Figure 4:



17.3 Additional Evidence on RIF's Potential for Treatment Shortening

17.3.1 Evidence from In Vitro Experiments

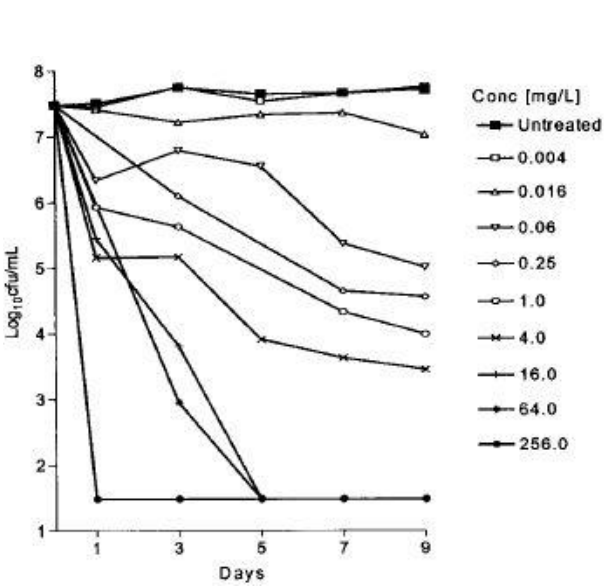
Since RIF's discovery as an orally bioavailable rifamycin,[117] much effort has been invested in identifying in vitro experimental conditions that predict *in vivo* efficacy. The minimum inhibitory concentration (MIC) of RIF for *M. tuberculosis* is 0.005 to 1.0 mcg/mL depending on the specific experimental conditions and the strain (including both wild-type *M. tuberculosis* and laboratory strains (i.e. H37Rv or H37Ra).[118-120] MICs on egg-based media (including Lowenstein-Jensen) are higher at 2.5-10 mcg/mL.[120, 121] The concentrations in non-egg based media are significantly lower than concentrations known to be effective *in vivo* and this large disparity may partially be explained by RIF's significant protein binding. Jayaram et al. reported that the MIC of RIF in *M. tuberculosis* strain H37Rv in broth (BACTEC 7H12B) alone was 0.1 mcg/mL. The authors attribute the higher MIC in the presence of serum to protein binding as the mean level of protein binding by RIF in these experiments (by equilibrium dialysis) was found to be 83.8%.[5] In light of the high level of protein binding found in humans (84-91%),[122] *in vitro* measurements derived in the absence of serum may significantly underestimate concentrations necessary for treatment in humans.

Higher concentrations of RIF may have favourable properties in addition to improved bactericidal activity, including prevention of resistance acquisition. Although the mutant prevention concentration of

RIF in vitro has been estimated at 2.2 mcg/mL,[123] Verbist et al reported that of 154 clinical isolates, 132 were susceptible to RIF at 2.5 mcg/mL, while all 154 were susceptible to RIF at 5 mcg/mL. And when *M. tuberculosis* H37Rv was exposed to RIF at concentrations from 2.5 to 80 mcg/mL, only the highest concentration suppressed all growth.[124]

Increasing the duration of exposure results in prolonged post-antibiotic effect (PAE).[18] Gumbo et al recently showed that the development of resistance and the length of the PAE may be related to the peak concentration of RIF *in vitro*. The emergence of resistance to RIF decreased over the range of C_{max}/MIC tested (from 0 to 250) and a 20-minute exposure to 14 mcg/mL yielded a longer PAE than longer incubations with lower concentrations of RIF.[98]

Ex vivo macrophage models suggest that elimination of intracellular infection may require higher concentrations of RIF than extracellular models.[125-127] In peritoneal macrophages isolated from BALB/c mice using *M. microti* as a model organism for *M. tuberculosis*, Dhillon and Mitchison found a 5-fold increase in MIC for RIF in mouse peritoneal macrophages as compared to cell-free media (0.05 mcg/mL versus 0.01 mcg/mL).[125] Furthermore, higher concentrations of RIF appear to yield better outcomes in intracellular models as well: using J774A.1 mouse macrophages, both time and concentration were shown to influence killing with a maximum reduction (of 2.3 $\log_{10}$ CFU) on day 4 with the maximum concentration of rifampin studied (50 mcg/mL).[5]



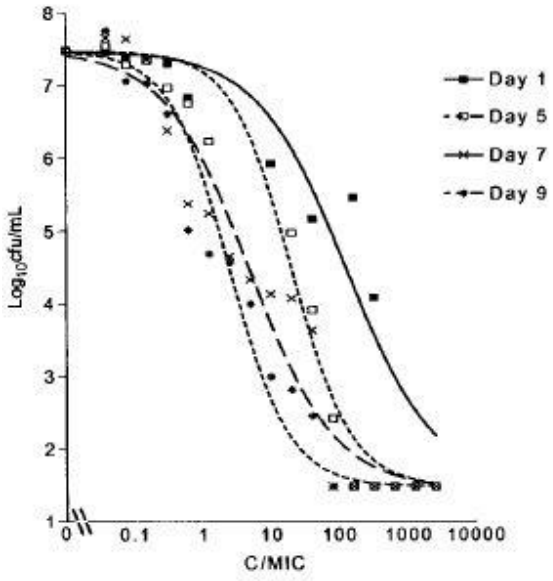


Figure 5: Effect of increasing Cmax/MIC ratios on the bactericidal activity of rifampin on days 1, 7, and 9 after the addition of drug (Jayaram et al)[5]

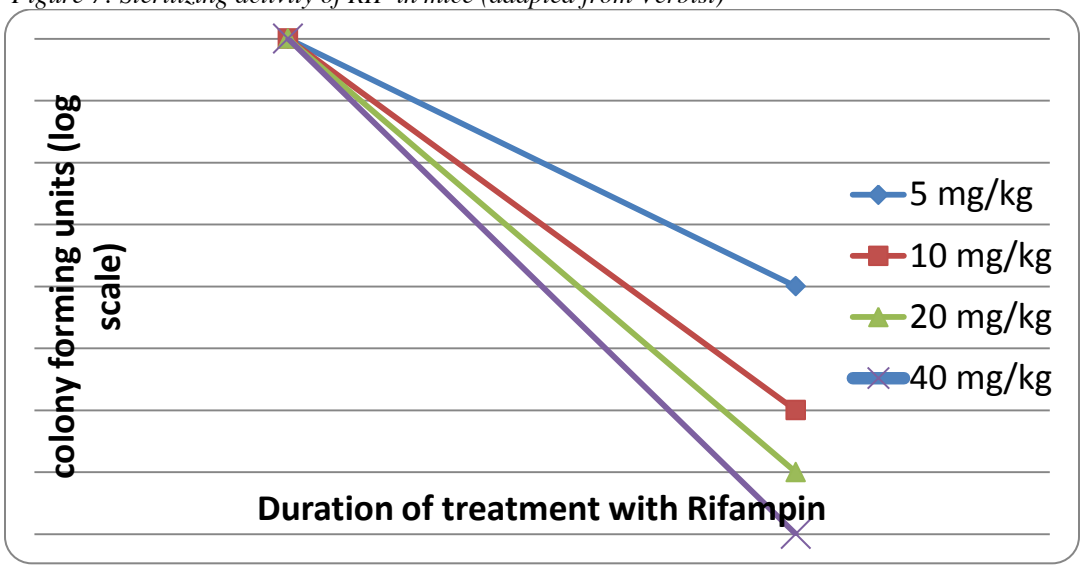
Figure 6: Growth of M. tuberculosis in BACTEC 7H12B broth following exposure to increasing concentrations of rifampin (Jayaram et al)[5]

17.3.2 Evidence from Animal Experiments

Work by Dickinson and Mitchison demonstrated the role of PZA and RIF in sterilizing activity in murine tuberculosis.[128] This was distinct from the contribution made by other drugs, such as INH and SM, to early killing. Grosset later affirmed these findings[129], illustrating that RIF acts both on rapidly multiplying organisms, and those persisters thought to be responsible for relapse.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Figure 7: Sterilizing activity of RIF in mice (adapted from Verbist)[22]



17.3.3 Evidence from Human Pharmacokinetics

RIF is a potent ligand for the orphan nuclear receptor PXR[130] resulting in potent induction of metabolism for many drugs.[131, 132] However, since RIF is not a substrate for CYP and its autoinduction does not appear to be mediated by these enzymes, the precise mechanism for autoinduction remains unknown. The half-life is unaffected by renal impairment but is increased by liver disease or biliary obstruction. RIF is deacetylated to the enterohepatically recirculated active metabolite 25-desacetyl rifampicin, and 50% to 60% is excreted in the feces. Up to 30% of a dose is excreted in the urine. Approximately 85% of circulating RIF is bound to plasma proteins, and is widely distributed throughout the body.[5, 122]

Table 10 *Limited change in time to full induction (and absolute amount of induction) with doubling of RIF dose*[29]

RIF dose	# of days	Timing	Antipyrine Clearance	% change	Antipyrine t1/2	% change
600 mg	7 days	Before	43.3		13.7	
		After	69.1	60	9.2	-33
1200 mg	7 days	Before	43.2		15.0	
		After	85.7	98	7.9	-47
600 mg	14 days	Before	37.4		11.2	
		After	79.7	113	6.1	-46

1200 mg	14 days	Before	49.6		11.3	
		After	101.1	104	6.0	-47

Substantial inter-patient variability in plasma concentrations of RIF (and rifapentine) has been documented repeatedly and low concentrations have been associated with decreased effectiveness of treatment.[31, 133-136] A suggested minimal target peak serum concentration for RIF is 8 mcg/mL and even this achieves a mean AUC/MIC of only 32.[16] Because only 15% of RIF plasma concentration is unbound,[5, 122] and because oral bioavailability is variable, higher doses increase serum concentrations[24, 30, 31] and are thought to produce more favourable penetration into all tissues, including large caseous or purulent lesions.[92, 135, 137, 138] This improved penetration and higher RIF concentrations at the site of infection may explain the observed enhanced sterilizing activity in animal models, and could improve outcomes in humans.[47]

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table 11 *Summary of safety, tolerability, and efficacy findings from clinical studies of high-dose RIF*

Authors, year (reference)	**Summary of Design**	**N***	**% Convert 1 month**	**% Convert 2 months**	**% failure &/or relapse**	**Adverse Events**
Decroix, Kreis, Sors et al, 1969[31]	**RIF (900 mg)** + H (750 mg) **twice weekly**	32	44	75	NA	None
	RIF (600 mg) + H (450 mg) **twice weekly**	38	39	70	NA	2 elevated LFT; 1 erythema
Kreis, Pretet, Birenbaum et al, 1976[139]$_1$	H (900mg) + SM (1 g) +					
	RIF 1200 mg (daily)	44	72.3	93.6	11.4	NA
	RIF 1200 mg (every 2 days)	47	70.4	93.2	14.9	
Long, Snider, Farer et al (USPHS), 1979[8]$_{2,3}$	I: H (300 mg) daily +					Leucopenia, hepatoxicity, arthritis, anemia (no significant different among groups)
	RIF (450 mg) daily, 20 weeks	167	24	60	22.4/3.1	6.2%
	RIF (600 mg) daily, 20 weeks	324	35	70	9.1/0.6	6.8%
	RIF (750 mg) daily, 20 weeks	331	33	75	9.4/0	5.7%
	C: H (300 mg) + EMB (15 mg/kg)					

						4% had drugs interrupted/terminated; 3 (0.05%) had RIF interrupted/terminated
E. Africa/BMRC, 1972[140]4	**Daily** SM (1 g) + H (300 mg), 6 months +	80	20	49	10/15	4%
	RIF (10mg/kg), 6 months	82	29	73	9/1	6%
	PZA (2 g), 6 months	73	29	64	10/6	9%
	THZ (150 mg), 6 months	66	17	46	8/16	8%
	SM, 8 weeks; THZ (150 mg), 18 months, daily	68	18	56	8/0	13%
Verbist & Rollier, 1971[135]	**RIF 30 mg/kg (1200, 1500, 1800 mg) +**					Transient increases in bilirubin only; renal monitoring, no AEs
	H (15 mg/kg)	53	NA	NA	NA	
	EMB (100 mg/kg)	43	NA	NA	NA	
Decroix, Kreis, Sors et al, 1971[52]5	**RIF (600 mg)** + H (450 mg) **daily**	47	37	71	0	2% changed due to AEs
	RIF (900 mg) + H (750 mg) **twice weekly**	50	44	74	0	1% changed due to AEs
Cooperative TB Chemotherapy Study, Poland, 1975[141]6	I: **RIF (600 mg)** + EMB (25 mg/kg) **daily** 12 weeks	247	25	60		Overall 12%: self-limiting increase in LFTs, asymptomatic. No permanent drug withdrawals.
	C: **RIF (1200 mg)** + EMB (50 mg/kg)					
	Once weekly, 12 months	123	NA	NA	2/6.7	29% systemic, 3% hepatic; rx withdrawn in 10%
	Twice weekly, 12 months	124	NA	NA	4/0.6	24% systemic, 2% hepatic; rx withdrawn in 10%

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Cooperative TB Chemotherapy Study, Poland, 1976[142] [6,7]	I: **RIF (600 mg)** + EMB (25 mg/kg) **daily,** 12 weeks					
	C: **RIF (600mg)** + EMB (50 mg/kg)				NA/6.7	
	Once weekly, 12 months	38	NA	NA	5/8	10% AEs; 2% treatment changed
	Twice weekly, 12 months	40	NA	NA	5/5	3% AEs; 0% treatment changed
	C: **RIF (1200 mg)** + EMB (50 mg/kg)		**NA**	**NA**	**2.9/0.6**	
	Once weekly, 12 months	35	NA	NA	4.6/0	33% AEs; 9% treatment changed
	Twice weekly, 12 months	30	NA	NA		
	Once weekly, 18 months	70	NA	NA	3.4/4	27% AEs; 19% treatment changed
	Twice weekly, 18 months		NA	NA		
	Once weekly, 24 months	68	NA	NA	2/0	26% AEs; 20% treatment changed
	Twice weekly, 24 months		NA	NA		
Hong Kong TB Treatment Services, 1975[143] H-Resistant, previously treated w/H, SM and/or PAS; adverse reactions evaluated up to 18 months[8]	I: EMB (15 mg/kg) **+ RIF (450 mg), daily,** 2 months					
	C: EMB (25 mg/kg) **+ RIF (450 mg), daily,** 16 months	42	NA	NA	2/0	11% of 47 (0 flu-like)
	C: EMB (25 mg/kg) **+ RIF (450 mg), daily,** 10 months	39	NA	NA	3/0	Excluded (not followed for 18 months)
	C: **RIF (>20 mg/kg)** + EMB (90 mg/kg) **once weekly**, 16 months	62	NA	NA	13	48% of 77 (40% flu-like)
	RIF (>20 mg/kg) + EMB (45 mg/kg) **twice weekly,** 18 months	84	NA	NA	21	32% of 68 (22% flu-like)
	RIF (>20 mg/kg) + EMB (90 mg/kg) **once weekly,** 18 months	53	NA	NA	19	61% of 72 (50% flu-like)
	I: Ethio (>10 mg/kg) + PZA (>30 mg/kg) + CS (0.5 g) daily,	68	NA	NA		54% of 57 (0 flu-like)

	6 months					
	C: Ethio (>10 mg/kg) + PZA (>30 mg/kg), daily, 12 months				19/0	
Dutt, Moers, Stead, 1983[61]9, elderly patients						Mean age of those experiencing AEs older than treatment population
	I: **RIF (600 mg)** + H (300 mg), **daily,** 1 month	586	NA	71	2.9/1.7	6% major (hepatitis, blood disorders); 0 flu-syndrome
	C: **RIF (600 mg)** + H (900 mg) **twice weekly** (8 months)					4.7% major (hepatitis, blood disorders); 1.5% flu-syndrome
Ruslami, Nijland, Alisjahbana et al, 2007[144]10	I: H (300 mg) daily + PZA (1500 mg) + EMB (750 mg) +		NA	NA		
	C: H (900 mg) **thrice weekly,** 3 months +					
	RIF (450 mg, 9.5 [1.4] mg/kg)	24			8	Hepatoxicity, grades 1 & 2: 20%; grade 3: 12%
	RIF (600 mg, 12.9 [1.7] mg/kg)	23			4	Hepatoxicity, grades 1 & 2: 46%; grade 3: 4%
Poole, Stradling, Worlledge, 1971[62]	I: SM (0.75 g) + H (300 mg) **+ RIF (600 mg), daily,** 3 months	37	NA	89		22% discontinued RIF: flu-like (16%); thrombocytopenia (6%)

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

	C: H (900 mg) **+ RIF (1200 mg), twice weekly**, 15 months				NA	Transient renal failure (1 patient)
Agarwal, 1983[145]	**RIF (450-2400 mg)** + SM (1 g) daily 1 month, **RIF (600-900 mg)** + H (15 mg) + SM weekly 2 months, H + SM weekly 2 months	30	33.3%	70.37%	NA	Jaundice, severe giddiness, flu-like symptoms

Footnotes:

NA= not reported or available.		*	Different numbers of subjects were used across analyses within studies. When possible, the N reported is the number of participants included in the analysis of the first endpoint.
		1	Failure= reappearance of bacilli and radiologic shadiness
Phase of Treatment		2	Failure= not culture converted on HR before 20 weeks. Also, after 20 weeks, while on HE: culture-positive, clinical non response, or prolongation of HE
I=Intensive	SM= streptomycin	3	Includes AEs in HE continuation phase
C=Continuation	THZ= thiacetazone	4	Failure= ≥ 1 positive culture in last 3 months of treatment
	RIF= rifampin/rifampicin	5	Failure= ≥ 1 positive (>+) culture in the last 6 months of treatment
	H=isoniazid	6	Failure= ≥ 2 positive cultures (≥6 results) at 44, 48, & 52 weeks, or in final 3 months of treatment
	EMB=ethambutol	7	This paper was the second report of the same study described in ref. 36. The design & results, specifically the randomization groups, were presented differently in the 2 reports. Moreover, although the 18-month and 24-month groups were split for dosing frequency (once or twice weekly), because no difference was detected, all results were aggregated by the study authors.
	Ethio=ethionamide	8	Failure= ≥ 2 positive cultures (among 6) at 16, 17, & 18 months
	CS=cycloserine	9	Failure= no bacteriologic conversion after 5-6 months of treatment
		10	Failure= Smear-positive at month 5 or later

17.3.3.1 *Pharmacogenomics Relevant to RIF Concentrations*

Recent unpublished work has revealed variability in RIF concentrations by polymorphisms in the SLCO1B1 gene, up to 35% decrease in AUC_{0-24}. The frequency of these polymorphisms varies by race.[146] Other preliminary work has suggested an association between other polymorphisms and hepatotoxicity induced by TB treatment [Davies GR, personal communication, 2009]. The ability to evaluate the effect of such polymorphisms on distribution of RIF concentrations will be important for interpretation of results.

17.3.4 Evidence from Efficacy Studies

Dozens of studies were undertaken during the 1960s-80s to evaluate RIF efficacy, toxicity, or tolerability, within a multidrug regimen. These are summarized in Table 11. Differences in design, dosing, and endpoints measured make formal comparison among the studies difficult, but certain patterns can be observed. We highlight the following findings. Decroix et al compared doses of 600 and 900 mg in combination with isoniazid. After two months, 75% of the 600 mg group and 30% of the 900 mg group had negative cultures. Earlier, the difference was larger: 19% at two weeks.[31] Another study compared two groups of patients that received 1200 mg RIF in combination with INH and streptomycin; in one arm dosage was daily, in the other intermittent.[139] The probability of culture conversion at one month was greater than 70% in both groups; at two months, the percent converted exceeded 93%. By comparison, in USPHS Study 19, only 60% of those receiving 450 mg of RIF, but approximately 70% of those receiving 600 mg/day and 75% of patients receiving 750 mg of RIF/day, had culture converted by 2 months.[8] In an East African/British MRC trial using approximately 10 mg/kg/day or RIF (450 mg in patients under 50 kg and 600 mg RIF in patients over 50 kg), results were similar to those found in the lower-dose arm in the USPHS study: at months one and two, 29% and 73% of patients respectively had negative cultures.[140] Looking informally across studies, the highest dose (1200 daily or intermittent) achieved 2-month conversion in approximately 20% more patients than did the middle doses (10mg/kg, 600 mg, or 750 mg) and 30% more than the lowest (450 mg). In sum, we observe that RIF doses higher than 10 mg/kg are associated with the frequency of culture conversion at both months 1 and 2.

17.4 Potential Risks: Additional Toxicity Evidence

17.4.1 Toxicity Data from Animals

Furesz et al reported evaluation of long-term toxicity (6 months) in rats, dogs, monkeys, and rabbits. In rats, doses up to 100 mg/kg revealed no toxicity, while doses of 200 mg/kg yielded some indications of hepatotoxicity. Mortality occurred in 1 dog at 25 mg/kg, with necrotic hemorrhagic enteritis. At 50 mg/kg, dogs showed some weight loss and hematologic changes. One dog died with severe renal and hepatic damage. There were weak indications of dose response in liver toxicity.[44] In monkeys, doses up to 75 mg/kg resulted in no toxicity. Vomiting and deterioration occurred among some of the animals at doses in excess of 105 mg/kg. Rabbits treated daily for 4 weeks tolerated 100 mg/kg with no symptoms. At 400 mg/kg, 3 of 5 rabbits died.

No evidence of ototoxicity or vestibular toxicity was found in mice or rabbits studied while receiving doses of 50 mg/kg or 150 mg/kg for 31 days.[147, 148] Although doses of 50 mg/kg and 100 mg/kg were not toxic to rat embryos, beginning at 200 mg/kg fetal death and abnormalities began to occur. All foetuses died at 300 mg/kg. Similar results were found among rabbits, though slight elevations in fetal abnormalities occurred at doses of 50 and 100 mg/kg. In teratogenicity studies, deformities occurred beginning at 150 mg/kg in mice and rats, but not in rabbits. Limited drug interaction studies were

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

performed in animals. In rats, 14-day studies of RIF (15 mg/kg or 30 mg/kg) with INH at 5 mg/kg revealed steatosis and liver cell damage in 7 of 31 rats. No fatty liver degeneration, however, was observed.[149]

17.4.2 Toxicity Data from Humans: Studies of Tuberculosis

Even non-linear increases in peak serum concentration (more than doubling for dose increase of 50%) do not appear to be associated with increased adverse event frequency.[31, 52] Although Verbist and Rollier observed transient increases in total and direct bilirubin in African patients receiving 30 mg/kg RIF (1200, 1500, 1800 mg doses) over 10 weeks, these increases did not persist and there were no additional signs or symptoms of liver toxicity in the trial, even among patients with baseline liver function abnormalities.[135] In one possible exception, Ruslami et al reported recently that grades 1 and 2 hepatotoxicity occurred more frequently in patients exposed to higher-dose RIF (p=0.054) while grade 3 hepatotoxicity occurred in 12% of the lower-dose and 4% of the higher-dose arm (p=0.32).[144]

Martinez and colleagues suggest that the increased frequency of flu-like syndrome in intermittent treatment is due to "an antibody excess relative to the antigen level in the drug-free days."[150] In at least one study, frequency of "flu-like" syndrome was higher in patients who received RIF (900-1800 mg) only once weekly when compared to patients who received it twice weekly.[143] Even then, these reactions may not warrant regimen changes[141] and typically do not occur until after three months of treatment. It is likely that any sterilizing and shortening effect of high-dose RIF would be realized in 8 weeks or less.[7, 8, 11, 12, 122] If higher doses of RIF were only used during the 8-week intensive phase, then existing data do not support increased frequency of these reactions.

Occasional thrombocytopenia, haemolytic anemia, and acute renal failure are associated with the presence of RIF-dependent IgM or IgG antibodies. RIF has variable effects on cellular and humoral immunity. Suppression of *in vitro* lymphocyte responses in cells collected from TB patients has been reported, but clinically evident immunosuppression has not been demonstrated.[16, 151] See Table 11 for summaries of additional studies.

17.4.3 Toxicity Data from Humans: Other Indications

With intermittent treatment for leprosy (usually at 900 mg) flu-like syndrome may be reported[66], but generally resolves spontaneously. RIF is also used commonly at 900 mg for 45-60 days to treat brucellosis. In combination with doxycycline, ROF has the "most favourable efficacy/safety ratio" among the recommended regiments[67] and was not associated with more adverse events than two other regiments in a multicentre trial.[68] RIF (20 mg/kg/day for 7 days) was used in an outbreak of resistant *Streptococcus pneumonia* at a day care in the US without any reports of adverse events.[69] In staphylococcal infections of orthopaedic implants, daily RIF (900 mg) was used in combination for 6 months with no treatment-related side effects reported in 46 patients.[70] RIF (1200 mg) was administered for 21 days in a patient with *Legionella jordanis* without any reported problem.[71] Lastly, in a randomized placebo-controlled trial, RIF was used at 1200 mg daily for 4 weeks to treat cutaneous leishmaniasis. The authors report that there were no elevations in liver function tests "or other side effects" during therapy.[72]

17.4.4 Toxicity of Companion Drugs

Isoniazid

Other nervous system reactions are rare at normal doses, and they include convulsions, encephalopathy, optic neuritis, memory impairment, and psychosis. Gastrointestinal adverse effects include nausea,

vomiting, and epigastric distress. Asymptomatic elevation of aminotransferases is common (10-20%). Idiosyncratic severe hepatitis is uncommon but more likely with increasing age (up to 2.3% incidence in persons more than 50 years old), and may be life threatening. The risk of isoniazid-associated hepatotoxicity is also increased by daily consumption of alcohol and in the postpartum period.

Pyrazinamide

In initial randomized clinical trials of a 2-month regimen of rifampin plus pyrazinamide (RZ) treatment for latent tuberculosis in HIV-infected persons, the incidence of severe liver injury was low and similar to that of daily isoniazid.[152-154] However, subsequent reports described severe liver injury in patients treated for latent tuberculosis infection with RZ, and on the basis of these reports, CDC collected data from cohorts of patients in the United States who received RZ for the treatment of LTBI during the period of January 2000 to June 2002. Of the 7.737 patients started on RZ, 77% received daily doses and 23% twice-weekly doses. A total of 204 patients (2.6%) discontinued treatment with RZ because of AST concentrations higher than five times the upper limit of normal. An additional 146 patients (1.9%) discontinued using RZ because of symptoms of hepatitis. Of the 30 patients (0.4%) with severe liver injury, 23 recovered and 7 died (0.09%). As a consequence, ATS and CDC recommends against routine use of RZ for treatment of latent TB infection. Several additional latent TB treatment studies confirm a relatively high frequency of hepatitis among RZ recipients—combined data from five recent studies include 1,311 patients showed a rate of Grade 3 or 4 liver injury of 5.8%, which is higher than that reported previously with isoniazid therapy.[155-159] In a randomized trial of two short-course regimens to prevent TB in high risk contacts of active TB cases in Rio de Janeiro, Brazil, Grade 3 or 4 liver injury developed in 17 of 163 (10%) participants receiving twice weekly RZ and <1% of participants receiving isoniazid plus rifapentine (RE Chaisson, unpublished data). The mechanism of RZ-associated hepatotoxicity remains unknown. Similarly, it is unclear why rates of hepatotoxicity with RZ for LTBI are higher than those observed among patients with active TB treated with isoniazid in addition to rifampin and pyrazinamide for 2 months. Possible explanations include impaired inflammatory response (resulting in impaired liver inflammation) in patients with active TB, or a mitigating effect of isoniazid on RZ-associated hepatotoxicity.

17.4.5 Drug Interactions between RIF and Companion Drugs

INH is largely cleared by N-acetyltransferase 2 (NAT2), which has not been shown to be induced by RIF.[160] Concurrent administration of larger doses of RPNT (cyclopentyl-RIF) did not alter the INH pharmacokinetics in USPHS Study 25.[85,161] PZA is metabolized to pyrazinoic acid and hydroxyl pyrazinoic acid by non-P450 pathways, and the metabolites are cleared renally.[25,162] EMB is partially cleared renally and partially cleared hepatically.[85,163] The precise enzymes involved in EMB metabolism have not been determined. Available data do not reveal differences in the pharmacokinetics of EMB when delivered alone compared to PK results for EMB in the presence of RIF.[25,164]

17.4.6 Potential interactions between companion medications and concomitant drugs

The companion drugs, INH, PZA and EMB, may also result in interactions with concomitant drugs. Implicated classes include anti-convulsants, sedatives, neuroleptics, anticoagulants, narcotics, anti-infectives, anti-gout, and antacids, in addition to several others. Details of the class members with interactions with companion drugs and suggestions for their management are listed below in Tables 12, 13 and 14. These drugs should be avoided in HIRIF patients, or management strategies should be followed.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table 12 *Drug-drug interactions with Isoniazid (likely in the HIRIF setting) requiring change of medication*[165]

Drug category	Drugs affected by Isoniazid	Comments
Anti-convulsants	Phenytoin, carbamazepine, valproic acid	Isoniazid decreases the clearance of these medications, and may increase the drug level in the blood. Blood levels of these drugs should be measured before and during treatment with INH. Additionally, the patient should be monitored closely for any sign or symptom of toxicity and the dose should be adjusted accordingly. Concomitant intake of phenytoin, carbamazepine and valproic acid may increase the hepatotoxicity of isoniazid.
	Phenobarbital	Concomitant use of phenobarbital with INH may lead to increased hepatotoxicity.
Sedatives	Benzodiazepines (i.e. diazepam, flurazepam, triazolam, midazolam)	INH may decrease the hepatic metabolism of benzodiazepines resulting in more elevated concentrations of these drugs in the blood. The patient should be observed closely for any sign of toxicity and the dose should be adjusted accordingly.
Neuroleptics	Chlorpromazine, haloperidol	Concomitant use of chlorpromazine with INH may compromise the metabolism of INH. These patients should be closely observed for INH toxicity. Concomitant use of haloperidol and INH may increase the level of haloperidol. Patients should be carefully monitored for possible haloperidol toxicity and the dose adjusted accordingly.
Anticoagulants	Coumadin and inadione derivatives (warfarin)	Concomitant use of these medications with INH may result in increased concentrations in plasma and thus increased risk of bleeding. Therefore, patients taking INH should be closely monitored.

Narcotics	Alfentanil, enflurane	Prolonged use of INH may increase duration of alfentanil in plasma; therefore the dose of this drug should be adjusted accordingly. INH may increase the formation of inorganic fluoride which may be nephrotoxic when used concomitantly with enflurane.
Other	Theophylline	Concomitant use of INH with this drug may result in increased level of theophylline in the blood, therefore theophylline levels should be monitored closely.
	Procainamide	Concomitant use of this drug with INH may result in increased plasma concentration of INH. These patients should be closely monitored for signs and symptoms of INH toxicity.
	Corticosteroids (e.g, prednisone)	In patients who are rapid acetylators, concomitant use of corticosteroids may decrease exposure to INH, and therefore doses of INH should be adjusted accordingly
	Acetaminophen, paracetamol	Concurrent use of these medications with INH may increase the risk of hepatotoxicity.
	Aluminum hydroxide	Interferes with INH absorption. While receiving INH therapy, acid suppressant drugs or antacids that do not contain aluminum hydroxide should be used.
	Disulfiram	Concomitant use of this medication with INH may increase the incidence of side effects of the central nervous system. Dosing for Disulfiram should be reduced or discontinued if necessary.
	Hepatotoxic medications	The use of INH with other hepatotoxic (non anti-TB) drugs should be avoided because they increase the possibility of hepatotoxicity.
	Neurotoxic medications	The use of INH with neurotoxic drugs should be avoided because may lead to an additive neurotoxic effect.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Table 13 *Drug-drug interactions with Pyrazinamide (likely in the HIRIF setting) requiring change of medication*[166]

Drug category	Drugs affected by pyrazinamide	Comments
Anti-infectives	Fluroquinolones	Concomitant use of Pyrazinamide and ofloxacin and Levofloxacin has been associated with an increase in adverse events (hepatic, gastrointestinal and musculoskeletal) resulting in discontinuation of therapy. Concomitant use of these medications is not recommended. However, when deemed necessary, it should be done under close medical vigilance. Patients will not be allowed to participate in HIRIF if on these drugs.
Anti-gout	Allopurinol	Allopurinol increases the AUC (area under the curve) of the pyrazinamide metabolite, pyrazinoic acid, which inhibits the elimination of urates. Therefore, allopurinol is not effective in treating hyperuricemia associated with pyrazinamide.
Other	Probenecid	There is a complex pharmacokinetic and pharmacodynamic interaction between these medications. An adequate dose of probenecid when used concomitantly with pyrazinamide has not been determined; therefore concomitant use should be avoided.
	Hepatotoxic medications	The use of pyrazinamide with other hepatotoxic (non anti-TB) drugs should be avoided because it increases the possibility of hepatotoxicity.

Table 14 *Drug-drug interactions with Ethambutol (likely in the HIRIF setting) requiring change of medication*[167]

Drug category	Drugs affected by Ethambutol	Comments
Antacids	Antacids containing aluminun hydroxide	While receiving ethambutol therapy, acid suppressant drugs or antacids that do not contain aluminum hydroxide should be used.
Anti-gout	Uricosurics	It may be necessary to increase the dose of uricosuric medications because ethambutol competes with uric acid for renal excretion.
	Disulfiram	Concomitant use of ethambutol with disulfiram may increase the risk for ocular toxicity.

17.5 Package Inserts

17.5.1 Rifadin

SUMMARY OF PRODUCT CHARACTERISTICS:

1. NAME OF THE MEDICINAL PRODUCT
Rifadin 150mg Capsules

2. QUALITATIVE AND QUANTITATIVE COMPOSITION
Rifampicin Ph Eur 150 mg
For a full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM
Capsule, hard.
The gelatine capsule is composed of a red half and a blue half.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications
Indications for use

Tuberculosis: In combination with other active anti-tuberculosis drugs in the treatment of all forms of tuberculosis, including fresh, advanced, chronic and drug resistant cases. Rifadin is also effective against most atypical strains of Mycobacteria.

Leprosy: In combination with at least one other active anti-leprosy drug in the management of multibacillary and paucibacillary leprosy to effect conversion of the infectious state to a non-infectious state.

Other Infections: In the treatment of Brucellosis, legionnaires Disease, and serious staphylococcal infections. To prevent emergence of resistant strains of the infecting organisms, Rifadin should be used in combination with another antibiotic appropriate for the infection.

Prophylaxis of meningococcal meningitis: For the treatment of asymptomatic carriers of *N. meningitides* to eliminate meningococci from the nasopharynx.

Haemophilus influenza: For the treatment of asymptomatic carriers of *H. influenza* and as chemoprophylaxis of exposed children, 4 years of age or younger.

4.2 Posology and method of administration
Recommended Dosage

For oral administration

The daily dose of Rifadin, calculated from the patient's body weight, should preferably be taken at least 30 minutes before a meal or 2 hours after a meal to ensure rapid and complete absorption.

Tuberculosis:
Rifadin should be given with other effective anti-tuberculosis drugs to prevent the possible emergence of rifampicin-resistant strains of Mycobacteria.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Adults: The recommended single daily dose in tuberculosis is 8-12 mg/kg.

Usual Daily dose: Patients weighing less than 50 kg – 450 mg. Patients weighing 50 kg or more – 600 mg.

Children: In children, oral doses of 10-20 mg/kg body weight daily are recommended, although a total daily dose should not usually exceed 600 mg.

Leprosy:
600 mg doses of rifampicin should be given once per month. Alternatively, a daily regimen may be used. The recommended single daily dose is 10 mg/kg.

Usual daily dose: Patients weighing less than 50 kg - 450 mg. Patients weighing 50 kg or more - 600 mg.

In the treatment of leprosy, rifampicin should always be used in conjunction with at least one other antileprosy drug,

Brucellosis, Legionnaires Disease or serious staphylococcal infections:

Adults: The recommended daily dose is 600-1200 mg given in 2 to 4 divided doses, together with another appropriate antibiotic to prevent the emergence of resistant strains of the infecting organisms.

Prophylaxis of meningococcal meningitis:
Adults: 600 mg twice daily for 2 days.

Children (1 - 12 years): 10 mg/kg twice daily for 2 days.

Children (3 months - 1 year): 5 mg/kg twice daily for 2 days.

Prophylaxis of Haemophilus influenza:

Adults and children: For members of households exposed to H. influenzae B disease when the household contains a child 4 years of age or younger, it is recommended that all members (including the child) receive rifampicin 20 mg/kg once daily (maximum daily dose 600 mg) for 4 days.
Index cases should be treated prior to discharge from hospital.

Neonates (1 month): 10 mg/kg daily for 4 days.

Impaired liver function:
A daily dose of 8 mg/kg should not be exceeded in patients with impaired liver function.

Use in the elderly:
In elderly patients, the renal excretion of rifampicin is decreased proportionally with physiological decrease of renal function; due to compensatory increase of liver excretion, the terminal half-life in serum is similar to that of younger patients.

RIFADIN 150mg label-EU. 2010 ed: Sanofi-Aventis S.p.A.

However, as increased blood levels have been noted in one study of rifampicin in elderly patients, caution should be exercised in using rifampicin in such patients, especially if there is evidence of impaired liver function.

4.3 Contraindications

Rifadin is contra-indicated in patients who:

- are hypersensitive to any of the rifamycins or any of the excipients (see section 6.1);
- have jaundice;
- are concurrently receiving saquinavir/ritonavir therapy (see section 4.5 Interactions).

4.4 Special warnings and precautions for use

Rifampicin should be given under the supervision of a respiratory or other suitably qualified physician.

Cautions should be taken in case of renal impairment if dose > 600 mg/day.

All tuberculosis patients should have pre-treatment measurements of liver function.

Adults treated for tuberculosis with rifampicin should have baseline measurements of hepatic enzymes, bilirubin, serum creatinine, a complete blood count, and a platelet count (or estimate).
Baseline tests are unnecessary in children unless a complicating condition is known or clinically suspected.

Patients with impaired liver function should only be given rifampicin in cases of necessity, and then with caution and under close medical supervision. In these patients, lower doses of rifampicin are recommended and careful monitoring of liver function, especially serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) should initially be carried out prior to therapy, weekly for two weeks, then every two weeks for the next six weeks. If signs of hepatocellular damage occur, rifampicin should be withdrawn.

Rifampicin should also be withdrawn if clinically significant changes in hepatic function occurs. The need for other forms of antituberculosis therapy and a different regimen should be considered. Urgent advice should be obtained from a specialist in the management of tuberculosis. If rifampicin is re-introduced after liver function has returned to normal, liver function should be monitored daily.
In patients with impaired liver function, elderly patients, malnourished patients, and possibly, children under two years of age, caution is particularly recommended when instituting therapeutic regimens in which isoniazid is to be used concurrently with Rifadin. If the patient has no evidence of preexisting liver disease and normal pre-treatment liver function, liver function tests need only be repeated if fever, vomiting, jaundice or other deterioration

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

in the patient's condition occur.

Patients should be seen at least monthly during therapy and should be specifically questioned concerning symptoms associated with adverse reactions.

In some patients hyperbilirubinaemia can occur in the early days of treatment. This results from competition between rifampicin and bilirubin for hepatic excretion.

An isolated report showing a moderate rise in bilirubin and/or transaminase level is not in itself an indication for interrupting treatment; rather the decision should be made after repeating the tests, noting trends in the levels and considering them in conjunction with the patient's clinical condition.

Because of the possibility of immunological reaction including anaphylaxis (see section 4.8 Undesirable effects) occurring with intermittent therapy (less than 2 to 3 times per week) patients should be closely monitored. Patients should be cautioned against interrupting treatment.

Rifampicin has enzyme induction properties that can enhance the metabolism of endogenous substrates including adrenal hormones, thyroid hormones and vitamin D. Isolated reports have associated porphyria exacerbation with rifampicin administration.

Rifadin capsules may produce a reddish coloration of the urine, sweat, sputum and tears, and the patient should be forewarned of this. Soft contact lenses have been permanently stained (see section 4.8).

All patients with abnormalities should have follow up examinations, including laboratory testing, if necessary.

4.5 Interaction with other medicinal products and other forms of interaction

Cytochrome P-450 enzyme interaction

Rifampicin is a potent inducer of certain cytochrome P-450 enzymes. Coadministration of rifampicin with other drugs that are also metabolised through these cytochrome P-450 enzymes may accelerate the metabolism and reduce the activity of these other drugs. Therefore, caution should be used when prescribing rifampicin with drugs metabolised by cytochrome P-450. To maintain optimum therapeutic blood levels, dosages of drugs metabolised by these enzymes may require adjustment when starting or stopping concomitantly administered rifampicin.
Examples of drugs metabolised by cytochrome P-450 enzymes are:

- Antiarrhythmics (e.g. disopyramide, mexiletine, quinidine, propafenone, tocainide),
- Antiepileptics (e.g. phenytoin),
- Hormone antagonist (antiestrogens e.g. tamoxifen, toremifene, gestinone),

- Antipsychotics (e.g. haloperidol, aripiprazole),
- Anticoagulants (e.g. coumarins),
- Antifungals (e.g. fluconazole, itraconazole, ketoconazole, voriconazole),
- Antivirals (e.g. saquinavir, indinavir, efavirenz, amprenavir, nelfinavir,
- atazanavir, lopinavir, nevirapine),
- Barbiturates
- Beta-blockers (e.g. bisoprolol, propanolol),
- Anxiolytics and hypnotics (e.g. diazepam, benzodiazepines, zolpicolone,
- zolpidem),
- Calcium channel blockers (e.g. diltiazem, nifedipine, verapamil,
- nimodipine, isradipine, nicardipine, nisoldipine),
- Antibacterials (e.g. chloramphenicol, clarithromycin, dapsone,
- doxycycline, fluoroquinolones, telithromycin),
- Corticosteroids
- Cardiac glycosides (digitoxin, digoxin),
- Clofibrate,
- Systemic hormonal contraceptives
- Oestrogen,
- Antidiabetic (e.g. chlorpropamide, tolbutamide, sulfonylureas,
- rosiglitazone),
- Immunosuppressive agents (e.g. ciclosporin, sirolimus, tacrolimus)
- Irinotecan,
- Thyroid hormone (e.g. levothyroxine),
- Losartan,
- Analgestics (e.g. methadone, narcotic analgesics),
- Praziquantel,
- Progestogens,
- Quinine,
- Riluzole,
- Selective 5-HT3 receptor antagonists (e.g. ondansetron
- Statins metabolised by CYP 3A4 (e.g. simvastatin),
- Theophylline,
- Tricyclic antidepressants (e.g. amitriptyline, nortriptyline),
- Cytotoxics (e.g. imatinib),
- Diuretics (e.g. eplerenone)

Patients on oral contraceptives should be advised to use alternative, nonhormonal methods of birth control during Rifadin therapy. Also diabetes may
become more difficult to control.

Other Interactions

When rifampicin is given concomitantly with the combination saquinavir/ritonavir, the potential for hepatotoxicity is increased. Therefore, concomitant use of Rifadin with saquinvir/ritonavir is contraindicated (see section 4.3 Contraindications).

RIFADIN 150mg label-EU. 2010 ed: Sanofi-Aventis S.p.A.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

When the two drugs were taken concomitantly, decreased concentrations of atovaquone and increased concentrations of rifampicin were observed.

Concurrent use of ketoconazole and rifampicin has resulted in decreased serum concentrations of both drugs.

Concurrent use of rifampicin and enalapril has resulted in decreased concentrations of enalaprilat, the active metabolite of enalapril. Dosage adjustments should be made if indicated by the patient's clinical condition.

Concomitant antacid administration may reduce the absorption of rifampicin. Daily doses of rifampicin should be given at least 1 hour before the ingestion of antacids.

When rifampicin is given concomitantly with either halothane or isoniazid, the potential for hepatotoxicity is increased. The concomitant use of rifampicin and halothane should be avoided. Patients receiving both rifampicin and isoniazid should be monitored closely for hepatotoxicity.

If *p*-aminosalicylic acid and rifampicin are both included in the treatment regimen, they should be given not less than eight hours apart to ensure satisfactory blood levels.

Interference with laboratory and diagnostic tests

Therapeutic levels of rifampicin have been shown to inhibit standard microbiological assays for serum folate and Vitamin B12. Thus alternative assay methods should be considered. Transient elevation of BSP and serum bilirubin has been reported. Rifampicin may impair biliary excretion of contrast media used for visualization of the gallbladder, due to competition for biliary excretion. Therefore, these tests should be performed before the morning dose of rifampicin.

4.6 Pregnancy and lactation

Pregnancy
At very high doses in animals rifampicin has been shown to have teratogenic effects. There are no well controlled studies with rifampicin in pregnant women. Although rifampicin has been reported to cross the placental barrier and appear in cord blood, the effect of rifampicin, alone or in combination with other antituberculosis drugs, on the human foetus is not known. Therefore, Rifadin should be used in pregnant women or in women of child bearing potential only if the potential benefit justifies the potential risk to the fetus. When Rifadin is administered during the last few weeks of pregnancy it may cause post-natal hemorrhages in the mother and infant for which treatment with Vitamin K1 may be indicated.

Lactation
Rifampicin is excreted in breast milk, patients receiving rifampicin should not breast feed unless in the physician’s judgment the potential benefit to the

patient outweighs the potential risk to the infant.

4.7 Effects on ability to drive and use machines

No studies on the effects on the ability to drive and use machines have been performed.

4.8 Undesirable effects

Reactions occurring with either daily or intermittent dosage regimens include:

Cutaneous reactions which are mild and self-limiting and do not appear to be hypersensitivity reactions. Typically they consist of flushing and itching with or without a rash. Urticaria and more serious hypersensitivity cutaneous reactions have occurred but are uncommon. Exfoliate dermatitis, pemphigoid reaction, erythema multiform including Stevens-Johnson syndrome, Lyells syndrome and vasculitis have been reported rarely.

Gastrointestinal reactions consist of anorexia, nausea, vomiting, abdominal discomfort and diarrhea. Pseudomembranous colitis has been reported with rifampicin therapy.

Hepatitis can be caused by rifampicin and liver function tests should be monitored (see section 4.4. Special warnings and precautions for use).

Central Nervous System: Psychoses have been rarely reported.

Thrombocytopenia with or without purpura may occur, usually associated with intermittent therapy, but is reversible if drug is discontinued as soon as purpura occurs. Cerebral hemorrhage and fatalities have been reported when rifampicin administration has been continued or resumed after the appearance of purpura.

Disseminated intravascular coagulation has also been rarely reported.

Eosinophilia, leucopenia, edema, muscle weakness and myopathy have been reported to occur in a small percentage of patients treated with rifampicin.
Agranulocytosis has been reported very rarely reported.
Rare reports of adrenal insufficiency in patients with compromised adrenal function have been observed.

Reactions usually occurring with intermittent dosage regimens and probably of immunological origin include:

- 'Flu Syndrome' consisting of episodes of fever, chills, headache, dizziness, and bone pain appearing most commonly during the 3rd to the 6th monthly of therapy. The frequency of the syndrome varies but may occur in up to 50 % of patients given once-weekly regimens with a dose of rifampicin of 25 mg/kg or more.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

- Shortness of breath and wheezing.
- Decrease in blood pressure and shock.
- Anaphylaxis.
- Acute hemolytic anemia.
- Acute renal failure usually due to acute tubular necrosis or acute interstitial nephritis.

If serious complications arise, e.g. renal failure, thrombocytopenia or hemolytic anemia, rifampicin should be stopped and never restarted.

Occasional disturbances of the menstrual cycle have been reported in women receiving long-term anti-tuberculosis therapy with regimens containing rifampicin.

Rifampicin may produce a reddish coloration of the urine, sweat, sputum and tears. The patient should be forewarned of this. Soft contact lenses may be permanently stained.

4.9 Overdose

Human Experience

Signs and Symptoms:
Nausea, vomiting, abdominal pain, pruritus, headache and increasing lethargy will probably occur within a short time after acute ingestion; unconsciousness may occur when there is severe hepatic disease. Transient increases in liver enzymes and/or bilirubin may occur. Brownish-red or orange coloration of the skin, urine, sweat, saliva, tears and feces will occur, and its intensity is proportional to the amount ingested. Facial or periorbital edema has also been reported in pediatric patients. Hypotension, sinus tachycardia, ventricular arrhythmias, seizures and cardiac arrest were reported in some fatal cases.
The minimum acute lethal or toxic dose is not well established. However, nonfatal acute overdoses in adults have been reported with doses ranging from 9 to 12 g rifampicin. Fatal acute overdoses in adults have been reported with doses ranging from 14-60 g. Alcohol or a history of alcohol abuse was involved in some of the fatal and nonfatal reports.
Nonfatal overdoses in pediatric patients ages 1 to 4 years old of 100 mg/kg for one to two doses have been reported.

Management:
Intensive supportive measures should be instituted and individual symptoms treated as they arise. Since nausea and vomiting are likely to be present, gastric lavage is probably preferable to induction of emesis. Following evacuation of the gastric contents, the instillation of activated charcoal slurry into the stomach may help absorb any remaining drug from the gastrointestinal

tract. Antiemetic medication may be required to control severe nausea and vomiting. Active diuresis (with measured intake and output) will help promote excretion of the drug. Hemodialysis may be of value in some patients.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: J04AB02 Antimycobacterials, antibiotics

Rifampicin is an active bactericidal antituberculosis drug which is particularly active against the rapidly growing extracellular organisms and also has bactericidal activity intracellularly. Rifampicin has activity against slow and intermittently-growing *M. Tuberculosis*.

Rifampicin inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme. Cross-resistance to rifampicin has only been shown with other rifamycins.

5.2 Pharmacokinetic properties

Rifampicin is readily absorbed from the gastrointestinal tract. Peak serum concentrations of the order of 10 µg/ml occur about 2 to 4 hours after a dose of 10 mg/kg body weight on an empty stomach.
Absorption of rifampicin is reduced when the drug is ingested with food.

The pharmacokinetics (oral and intravenous) in children are similar to adults.

In normal subjects the biological half-life of rifampicin in serum averages about 3 hours after a 600 mg dose and increases to 5.1 hours after a 900 mg dose. With repeated administration, the half-life decreases and reaches average values of approximately 2-3 hours. At a dose of up to 600 mg/day, it does not differ in patients with renal failure and consequently, no dosage adjustment is required.

Rifampicin is rapidly eliminated in the bile and an enterophepatic circulation ensues. During this process, rifampicin undergoes progressive deacetylation, so that nearly all the drug in the bile is in this form in about 6 hours. This metabolite retains essentially complete antibacterial activity. Intestinal reabsorption is reduced by deacetylation and elimination is facilitated. Up to 30 % of a dose is excreted in the urine, with about half of this being unchanged drug.

Rifampicin is widely distributed throughout the body. It is present in effective concentrations in many organs and body fluids, including cerebrospinal fluid. Rifampicin is about 80 % protein bound. Most of the unbound fraction is not ionized and therefore is diffused freely in tissues.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

5.3 Preclinical safety data

Not applicable

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Corn starch Ph Eur
Magnesium stearate Ph Eur

6.2 Incompatibilities

None stated

6.3 Shelf life

4 years from date of manufacture

6.4 Special precautions for storage

Store below 25oC.
Protect from light and moisture.

6.5 nature and contents of container

Amber glass bottles of 100 capsules.

Blister packs of 100 capsules in cardboard cartons. Blister material is aluminum foil / PVDC (Aluminium 0.025 mm; PVDC 20 gsm) and transparent PVC / PVDC foil (PVC 0.25 mm; PVDC 60 gsm).

6.6 Special precautions for disposal

No special requirements.

7. MARKETING AUTHORISATION HOLDER

Aventis Pharma Limited
Trading as Marion Merrell or Aventis Pharma
One Onslow Street
Guildford
Surrey
GU1 4YS
UK

Or trading as

Sanofi-aventis
One Onslow Street

Guildford
Surrey
GU1 4YS
UK

8. MARKETING AUTHORISATION NUMBER(S)

PL 04425/5915R

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

9th April 2005

10. DATE OF REVISION OF THE TEXT

18 November 2011

LEGAL CLASSIFICATION

POM

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

17.5.2 FDCs

Products: 4 FDC-B and 4 FDC

RIFAMPICIN 150 mg, ISONIAZID 75 mg, PYRAZINAMIDE 400 mg and ETHAMBUTOL 275 mg in a fixed dose combination tablet

Uses:
An optional component of several anti-TB chemotherapeutic regimens currently recommended by WHO.

Contraindications and Precautions:
•Preparation not suitable for use in children
•Not appropriate for intermittent therapy in these drug strengths
•Known hypersensitivity to the drugs

See also individual monographs for Rifampicin, Isoniazid, Pyrazinamide and Ethambutol below:

Dose:
Use for initial (intensive) phase of treatment in place of the single tablets: *By mouth*, **ADULT**
•30-37 kg 2 tablets daily for 2 months
•38-54 kg 3 tablets daily for 2 months
•55-70 kg 4 tablets daily for 2 months
•71 kg or more 5 tablets daily for 2 months

RIFAMPICIN

General information

A semisynthetic derivative of rifamycin, a complex macrocyclic antibiotic that inhibits ribonucleic acid synthesis in a broad range of microbial pathogens. It has bactericidal action and a potent sterilizing effect against tubercle bacilli in both cellular and extracellular locations. Rifampicin is lipid-soluble. Following oral administration, it is rapidly absorbed and distributed throughout the cellular tissues and body fluids; if the meninges are inflamed, significant amounts enter the cerebrospinal fluid. A single dose of 600 mg produces a peak serum concentration of about 10 micrograms/ml in two to four hours, which subsequently decays with a half-life of two to three hours. It is extensively recycled in the enterohepatic circulation, and metabolites formed by deacetylation in the liver are eventually excreted in the feces. Since resistance readily develops, rifampicin must always be administered in combination with other effective antimycobacterial agents.

Clinical information

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

Uses

A component of all six and eight month anti-TB chemotherapeutic regimens currently recommended by WHO; Leprosy

Dosage and administration

Rifampicin should preferably be given at least 30 minutes before meals, since absorption is reduced when it is taken with food. Rifampicin should be given as combination therapy Adults and children: 10 mg/kg daily, *or* 2 or 3 times weekly (maximum dose, 600 mg daily)

Contraindications

Known hypersensitivity to rifamycins
Jaundice

Precautions

Serious immunological reactions resulting in renal impairment, hemolysis or thrombocytopenia are on record in patients who resume taking rifampicin after a prolonged lapse of treatment. In this rare situation it should be immediately and definitely withdrawn.

Careful monitoring of liver function is required in the elderly, and in patients who are alcohol-dependent, have hepatic disease or are on prolonged therapy.

Reduce dose in renal impairment.

Patients should be warned that treatment may produce reddish coloration of urine, tears, saliva and sputum, and that contact lenses may be irreversibly stained.

Patients or their care-givers should be told how to recognize signs of liver disorders and advised to discontinue treatment and seek immediate medical attention if symptoms such as persistent nausea, vomiting, malaise or jaundice develop.

Use in pregnancy

Whenever possible, the six month regimen based upon isoniazid, rifampicin and pyrazinamide should be used. Vitamin K should be administered to the infant at birth because of the risk of postnatal hemorrhage.

Adverse effects

Rifampicin is well tolerated by most patients at currently recommended doses, although gastrointestinal tolerance can be unacceptably severe. Other adverse effects (skin rashes, fever, influenza-like syndrome and thrombocytopenia) are more likely to occur with intermittent administration. Exfoliative dermatitis is more frequent in HIV-positive TB patients. Temporary oliguria, dyspnea and hemolytic anemia have also been reported in patients taking the drug three times weekly. These reactions usually subside if the regimen is changed to one with daily dosage. Moderate rises in serum concentrations of bilirubin and transaminases, which are common at the outset of treatment, are often transient and without clinical significance. However, dose-related hepatitis can occur which is potentially fatal. It is consequently important not to exceed the maximum recommended daily dose of 10 mg/kg (600 mg).

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

Drug interactions

Rifampicin induces hepatic enzymes, and may increase the dosage requirements of drugs metabolized in the liver. These include corticosteroids, steroid contraceptives, oral hypoglycemic agents, oral anticoagulants, phenytoin, cimetidine, cyclosporin and digitalis glycosides. Since rifampicin reduces the effectiveness of the oral contraceptive pill, women should consequently be advised to choose between one of the following two options for contraception. Following consultation with a physician, she could take an oral contraceptive pill containing a higher dose of estrogen (50mcg). Alternatively she could use a nonhormonal method of contraception throughout rifampicin treatment and for at least one month subsequently.

ISONIAZID

General information

Isoniazid, the hydrazide of isonicotinic acid is highly bactericidal against replicating tubercle bacilli. It is rapidly absorbed and diffuses readily into all fluids and tissues. The plasma half-life, which is genetically determined, varies from less than one hour in fast acetylators to more than three hours in slow acetylators. It is largely excreted in the urine within 24 hours, mostly as inactive metabolites.

Clinical information

Uses

Tuberculosis treatment, in combination with other drugs

Tuberculosis prophylaxis and occasionally to prevent:
• transmission to close contacts at high risk of disease
• progression of infection to primary complex in recently infected, asymptomatic individuals
• recrudescence of infection in immunodeficient individuals.

Dosage and administration
By mouth, ADULT and CHILD
•5 mg/kg (4–6 mg/kg) daily (maximum, 300 mg daily)
•*or* 10 mg/kg 3 times weekly
•*or* 15 mg/kg twice weekly

Tuberculosis, treatment in critically ill patients unable to take oral therapy (combination therapy), *use intramuscular injection* , **ADULT** 200–300 mg as single daily dose; **CHILD** 10– 20 mg/kg daily

Tuberculosis, prophylaxis, *by mouth*
•**ADULT** 300 mg daily for at least 6 months
•**CHILD** 5 mg/kg daily for at least 6 months

Note: Isoniazid should be taken on an empty stomach; if taken with food to reduce gastrointestinal irritation, oral absorption and bioavailability may be impaired

Contraindications
• Known hypersensitivity
• Drug induced hepatic disease

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

Precautions

• Hepatic impairment (monitor hepatic function)
• Malnutrition
• Chronic alcohol dependence
• Chronic renal failure
• Diabetes mellitus
• HIV infection—prophylactic pyridoxine 10 mg daily required because risk of peripheral neuritis
• Epilepsy (isoniazid may provoke attacks)
• Slow acetylator status (increased risk of adverse effects)
• History of psychosis
• Pregnancy
• Breast-feeding
• Porphyria

Note: Patients at risk of peripheral neuropathy as a result of malnutrition, chronic alcohol dependence or diabetes should additionally receive pyridoxine, 10 mg daily.

Note: For liver disorders, patients or their care-givers should be told how to recognize signs of liver disorder, and advised to discontinue treatment and seek immediate medical attention if symptoms such as nausea, vomiting, malaise or jaundice develop

Adverse effects

Isoniazid is generally well tolerated at recommended doses. Systemic or cutaneous hypersensitivity reactions occasionally occur during the first weeks of treatment. The risk of peripheral neuropathy is excluded if vulnerable patients receive daily supplements of pyridoxine. Other less common forms of neurological disturbance, including optic neuritis, toxic psychosis and generalized convulsions, can develop in susceptible individuals, particularly in the later stages of treatment and occasionally necessitate the withdrawal of isoniazid. Hepatitis is an uncommon but potentially serious reaction that can usually be averted by prompt withdrawal of treatment. More often, however, a sharp rise in serum concentrations of hepatic transaminases at the outset of treatment is not of clinical significance, and usually resolves spontaneously during continuation of treatment.

Drug interactions

Isoniazid tends to raise plasma concentrations of phenytoin and carbamazepine by inhibiting their metabolism in the liver. The absorption of isoniazid is impaired by aluminium hydroxide.

Overdosage

Nausea, vomiting, dizziness, blurred vision and slurring of speech occur within 30 minutes to three hours of overdosage. Massive poisoning results in coma preceded by respiratory depression and stupor. Severe intractable seizures may occur. Emesis and gastric lavage can be of value if instituted within a few hours of ingestion. Subsequently, hemodialysis may be of value. Administration of high doses of pyridoxine is necessary to prevent peripheral neuritis.

Storage

Tablets should be kept in well-closed containers, protected from light. Solution of injection should be stored in ampoules protected from light.

PYRAZINAMIDE

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

General information

A synthetic analogue of nicotinamide that is only weakly bactericidal against M. tuberculosis, but has potent sterilizing activity, particularly in the relatively acidic intracellular environment of macrophages and in areas of acute inflammation. It is highly effective during the first two months of treatment while acute inflammatory changes persist and its use has enabled treatment regimens to be shortened and the risk of relapse to be reduced.

It is readily absorbed from the gastrointestinal tract and is rapidly distributed throughout all tissues and fluids. Peak plasma concentrations are attained in two hours and the plasma half-life is about 10 hours. It is metabolised mainly in the liver and is excreted largely in the urine.

Clinical information

Uses

A component of all six and eight month anti-TB chemotherapeutic regimens currently recommended by WHO.

Dosage and administration

By mouth:

Adults and children (for the first two or three months) 25 mg/kg daily 35 mg/kg three times weekly 50 mg/kg two times weekly

Contraindications

•Known hypersensitivity
•Severe hepatic impairment
•Porphyria

Precautions

•Hepatic impairment (monitor hepatic function)
•Patients with diabetes mellitus (monitored blood glucose--concentrations may change suddenly
•Gout may be exacerbated.

Use in pregnancy

Although the safety of pyrazinamide in pregnancy has not been established, the six month regimen based upon isoniazid, rifampicin and pyrazinamide should be used whenever possible.

Adverse effects

Pyrazinamide in usually well tolerated. Hypersensitivity reactions are rare, but some patients complain of slight flushing of the skin.

Moderate rises in serum transaminase concentrations are common during the early phases of treatment. Severe hepatotoxicity is rare.
As a result of inhibition of renal tubular secretion, a degree of hyperuricemia usually occurs, but this is often asymptomatic. Gout requiring treatment with allopurinol occasionally develops. Arthralgia, particularly of the shoulders, commonly occurs and is responsive to simple analgesics. Both

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

hyperuricemia and arthralgia may be reduced by prescribing regimens with intermittent administration of pyrazinamide.

Note: Patients and their care-givers should be told how to recognize signs of liver disorder, and advised to discontinue treatment and seek immediate medical attention if symptoms such as persistent nausea, vomiting, malaise or jaundice develop.

Overdosage

Little had been recorded on the management of pyrazinamide overdose. Acute liver damage and hyperuricemia have been reported. Treatment is essentially symptomatic. Emesis and gastric lavage may be of value if undertaken within a few hours of ingestion. There is no specific antidote and treatment is supportive.

Storage

Tablets should be stored in tightly closed containers, protected from light.

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

ETHAMBUTOL

General information

A synthetic congener of 1,2-ethanediamine that is active against M. tuberculosis, M. bovis and some non-specific mycobacteria. It is used in combination with other anti-TB drugs to prevent or delay the emergence of resistant strains.

It is readily absorbed from the gastrointestinal tract. Plasma concentrations peak in 2-four hours and decay with a half-life of three to four hours. Ethambutol is excreted in the urine both unchanged and as inactive hepatic metabolites.

About 20% is excreted in the feces as unchanged drug.

Clinical information

Uses

An optional component of several anti-TB chemotherapeutic regimens currently recommended by WHO.

Dosage and administration

By mouth: Adults: 15 mg/kg daily 30 mg/kg three times weekly, or 45 mg/kg (40-50 mg/kg) twice a week

Children: 15 mg/kg daily

Dosage must always be carefully calculated on a weight basis to avoid toxicity, and should be reduced in patients with impaired renal function.

Contraindications

- Known hypersensitivity
- Pre-existing optic neuritis from any cause
- Inability to report symptomatic visual disturbances—children under 5 years)
- Severe renal impairment

Precautions

- Visual disturbances—ocular examination recommended before and during treatment (see note below)
- Reduce dose in renal impairment and monitor plasma concentration
- Use in the elderly

Note: Patients should report visual disturbances immediately and discontinue treatment; children who are incapable of reporting symptomatic visual changes accurately should be given alternative therapy, as should, if possible, any patient who cannot understand

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

warnings about visual adverse effects

Whenever possible, renal function should assessed before treatment.

Use in pregnancy

The six month regimen based upon isoniazid, rifampicin and pyrazinamide should be used. If a fourth drug is needed during the initial phase, ethambutol should be preferred to streptomycin.

Adverse effects

Dose-dependent optic neuritis can readily result in impairment of visual acuity and color vision. Early changes are usually reversible, but blindness can occur if treatment is not discontinued promptly.

Signs of peripheral neuritis occasionally develop in the legs.

Overdosage

Emesis and gastric lavage may be of value if undertaken within a few hours of ingestion. Subsequently, dialysis may be of value. There is no specific antidote and treatment is supportive.

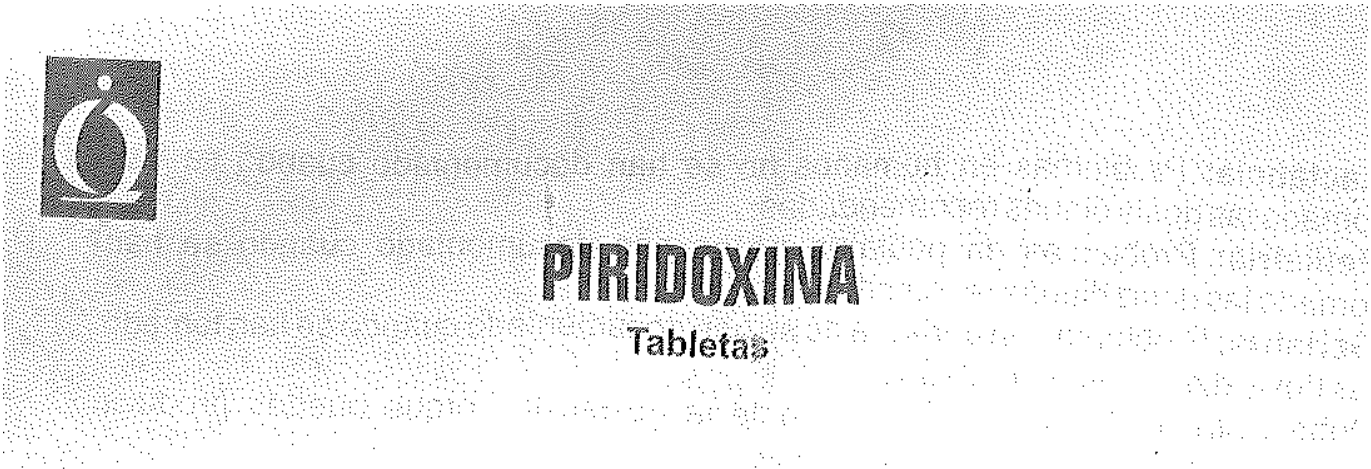
Storage

Tablets should be stored in well-closed containers.

FDC label/patient leaflet, Macleods Pharmaceuticals Ltd, WHO prequalification.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

17.5.3 Pyridoxine



PIRIDOXINA

Tabletas

COMPOSICION

Cada tableta contiene:

Piridoxina Clorhidrato ..... 50 mg

Excipientes c.s.p.

ACCION FARMACOLOGICA

- Piridoxina es convertida en los eritrocitos en piridoxal fosfato y, en una menor medida a piridoxamina fosfato, los cuales actúan como coenzima para diversas funciones metabólicas afectando la utilización de proteínas, carbohidratos y lípidos. La Piridoxina está involucrada en la conversión de triptófano en niacina o serotonina, cambio de glucógeno a glucosa-1-fosfato, conversión de oxalato a glicina, síntesis de ácido gamma aminobutírico (GABA) dentro del SNC, y síntesis de heme.
- La Piridoxina incrementa la excreción de ciertas drogas (por ejemplo: cicloserina e isoniazida) que actúan como antagonistas de la Piridoxina.

126

Pyridoxine 50mg. IQFARMA. Instituto Quimioterápico S.A.

INDICACIONES

- Prevención y tratamiento de los estados de deficiencia de Piridoxina. La deficiencia de Piridoxina puede ocurrir como resultado de una inadecuada nutrición o mala absorción intestinal.
- Se puede requerir suplementos de Piridoxina en las siguientes condiciones (basados en deficiencias de Piridoxina): Alcoholismo, quemaduras, disfunción metabólica congénita, falla cardiaca congestiva, fiebre crónica, gastrectomía, hemodiálisis, hipertiroidismo, infección, enfermedades intestinales (diarrea, enteritis regional, esprue), síndrome de mala absorción asociada con enfermedad del tracto hepático-biliar, como el alcoholismo con cirrosis.
- En algunas dietas inusuales que no aportan los requerimientos mínimos de Piridoxina.
- Durante el embarazo y lactancia se recomienda incrementar la ingesta de todas las vitaminas y la mayoría de minerales.
- Se recomienda incrementar la ingestión de Piridoxina cuando se está recibiendo: cicloserina, etionamida, hidralazina, inmunosupresores, isoniazida, penicilamina y anticonceptivos orales que contienen estrógeno.
- Tratamiento de la intoxicación con cicloserina, o isoniazida, se usa como antídoto en el envenenamiento con cicloserina y para eliminar las convulsiones y prevenir la neuropatía asociada al envenenamiento con isoniazida.

INTERACCIONES

- Levodopa: Su uso concomitante con Piridoxina no se recomienda, desde que los efectos antiparkinsonianos de la Levodopa son revertidos con pequeñas dosis como de 5 mg de Piridoxina oralmente.
- Dependiendo de la cantidad presente se ha observado interacciones con cicloserina, etionamida, hidralazina, inmunosupresores (azatioprina, clorambucil, corticosteroides, corticotropina (ACTH), ciclofosfamida, ciclosporina, mercaptopurina), isoniazida, penicilamina, estrógenos, o anticonceptivos que contienen Estrógenos.

CONTRAINDICACIONES

Se debe evaluar el riesgo/ beneficio cuando existan los siguientes problemas médicos:

- Hipersensibilidad a la Piridoxina.

PRECAUCIONES

- Embarazo: Problemas en humanos no se han documentado cuando se han tomado las dosis diarias recomendadas.

Pyridoxine 50mg. IQFARMA. Instituto Quimioterápico S.A.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

- Lactancia: Problemas en humanos no se han documentado cuando se han tomado las dosis diarias recomendadas.
- Pediatría: Problemas en pediatría no se han documentado cuando se han tomado las dosis diarias recomendadas.
- Geriatría: Problemas en geriatría no se han documentado cuando se han tomado las dosis diarias recomendadas.
- Debe evitarse dosis excesivas durante la gestación porque puede producir síndrome de dependencia en el neonato.
- No se ha demostrado la efectividad de piridoxina para el tratamiento de acné y otras dermatosis, intoxicación alcohólica, asma, hemorroides, cálculos renales, desórdenes mentales, cefaleas, migrañas, estimulación del apetito, dolor miofacial y neuropatía.

REACCIONES ADVERSAS

- Dosis de 200mg/día por más de 30 días han reportado que produce síndrome de dependencia a la Piridoxina.
- Altas dosis de Piridoxina (2 a 6 g/día) ingeridos por varios meses han causado neuropatías sensoriales severas.

ADVERTENCIAS

- No usar las vitaminas como sustitutos de una dieta balanceada
- Manténgase alejado de los niños.
- Proteger de la luz.

Pyridoxine 50mg. IQFARMA. Instituto Quimioterápico S.A.

DOSIS Y VIA DE ADMINISTRACIÓN

Dosis usual en el adulto y adolescente

Prevención de la deficiencia: Las cantidades están basadas sobre una ingesta diaria normal recomendada.

- **Adolescentes y adultos varones:** 1,7 a 2 mg
- **Adolescentes y adultos mujeres:** 1,4 a 1,6 mg
- **Mujeres gestantes** : 2,2 mg
- **Mujeres que dan de lactar** : 2,1 mg

Tratamiento de la deficiencia: La dosis debe ser individualizada por el médico de acuerdo a la severidad de la deficiencia.

Dosis usual pediátrica

Prevención de la deficiencia: Las cantidades están basadas sobre una ingesta diaria normal recomendada.

- **Recién nacidos hasta los 3 años de edad** : 0,3 a 1 mg.
- **Niños de 4 a 6 años de edad** : 1,1 mg.
- **Niños de 7 a 10 años de edad** : 1,4 mg.

Tratamiento de la deficiencia: La dosis debe ser individualizada por el médico de acuerdo a la severidad de la deficiencia.

Vía de administración: Oral.

TRATAMIENTO EN CASO DE SOBREDOSIS

- No se han reportado casos de hipervitaminosis o intoxicación con respecto a esta vitamina. De llegar a ocurrir una sobredosis, el tratamiento deberá ser sintomático y de soporte.

CONDICIONES DE ALMACENAMIENTO

- Mantener el producto en lugar fresco y seco, a temperatura entre 15ºC y 30ºC, protegido de la luz.

130109

IQFARMA
Instituto Quimioterápico S.A.
E-mail: infomedic@iqfarma.com
Av. Santa Rosa 350 - Santa Anita, Lima - Perú
☎ 612-0707

Pyridoxine 50mg. IQFARMA. Instituto Quimioterápico S.A.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

17.6 Adverse Event grading

Figure 8. Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table, November 2007, Draft

Note: The following toxicity table is a DRAFT and designed to provide general guidance on parameters for monitoring safety in clinical trials. This toxicity table is not comprehensive and should not be applied directly to all trials.

When selecting a toxicity table, the following are some of the items that must be taken into consideration:

- The population being studied
  - Does the clinical trial evaluate healthy subjects, subjects with a particular disease or condition?
- The stage of test article development
  - Is the clinical trial a Phase I, II, III or IV?
- The type of test article
  - Does the clinical trial evaluate a drug, device, vaccine or other biologic agent?
- The prior human and preclinical experience with the test article
  - Are there any specific findings that require adjustment of the toxicity table?

Single site clinical trials evaluating healthy subjects should conform to the laboratory normal values at the single site. Multi-center clinical trials should reconcile among their laboratory normal values when evaluating a healthy volunteer population.

Please confer with the DMID protocol team and DMID's Office of Clinical Research Affairs when selecting or developing a toxicity table for a DMID-sponsored trial.

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal
R_x = Therapy	Req = Required
Mod = Moderate	IV = Intravenous
ADL = Activities of Daily Living	Dec = Decreased

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild**	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	**Moderate**	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
GRADE 4	**Life-threatening**	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supercede the use of these tables for specified criteria.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	6.5 - 7.9 gm/dL	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/mm^3	750-999/mm^3	500-749/mm^3	<500/mm^3
Platelets	75,000-99,999/mm^3	50,000-74,999/mm^3	20,000-49,999/mm^3	<20,000/mm^3
WBCs	11,000-13,000/mm^3	13,000-15,000/mm^3	15,000-30,000/mm^3	>30,000 or <1,000/mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	---------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL ---------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20.0 %

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

CHEMISTRIES				
	Grade 1	Grade 2	Grade 3	Grade 4
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE
NOVEMBER 2007
DRAFT

CHEMISTRIES (continued)				
	Grade 1	Grade 2	Grade 3	Grade 4
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	> 1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	12.1 – 15.0 mg/dL	>15.0 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

ENZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN

URINALYSIS				
	Grade 1	Grade 2	Grade 3	Grade 4
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

RESPIRATORY				
	Grade 1	Grade 2	Grade 3	Grade 4
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring Oxygen therapy

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE
NOVEMBER 2007
DRAFT

MUSCULOSKELATEL				
	Grade 1	Grade 2	Grade 3	Grade 4
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity	frank myonecrosis

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007 DRAFT

SKIN				
	Grade 1	Grade 2	Grade 3	Grade 4
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm	
Erythema	< 15mm	15-30 mm	>30mm	
Edema	< 15mm	15-30 mm	>30mm	
Rash at Injection Site	< 15mm	15-30 mm	>30mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

17.7 Point Estimates and Confidence Intervals for Frequency of AEs or SAEs

Below we present point estimates and 80% and 90% binomial confidence intervals (CIs) for rates of AEs/SAEs. These represent the frequency that will be observed when the study recruitment is completed. Since the events will likely occur prior to the end of recruitment, the observed frequency of toxicity at any point during the study will be higher than this, but these estimates provide, at least, a lower bound.

We provide CIs with 80% and 90% confidence around point estimates of frequency of AEs/SAEs in all arms (Table 15) and frequency of AEs/SAEs in a single arm (Table 16). In each table, we also provide estimates for the overall study (n=180) and estimates for single arms (as if data were unblended and we knew in which arm events occurred).

Table 15 *Point estimates and binomial confidence intervals of toxicity in HIRIF, full study (remains blinded)*

Observed events, N	Point Estimate	Lower 80% CI	Upper 80% CI
1,180	0.005555556	0.0005851649	0.0214368369
2,180	0.01111111	0.002958368	0.029296637
3,180	0.01666667	0.006137957	0.036737262
4,180	0.02222222	0.009727259	0.043917538
5,180	0.02777778	0.01357388	0.05091537
Observed events, N	Point Estimate	Lower 90% CI	Upper 90% CI
1,180	0.005555556	0.0002849221	0.0260823033
2,180	0.01111111	0.00197778	0.03456159
3,180	0.01666667	0.004557748	0.042510022
4,180	0.02222222	0.007625414	0.050128307
5,180	0.02777778	0.01100769	0.05751495

Table 16 *Point estimates and binomial confidence intervals of toxicity in HIRIF, single arm (if unblinded, and could know distribution within arms)*

Observed events, n	Point Estimate	Lower 80% CI	Upper 80% CI
1,60	0.01666667	0.001754468	0.063287383
2,60	0.03333333	0.008898553	0.086278042
3,60	0.05	0.01850794	0.10796524
Observed events, n	Point Estimate	Lower 90% CI	Upper 90% CI
1,60	0.01666667	0.0008545229	0.0766399949
2,60	0.03333333	0.005954897	0.101235895
3,60	0.05	0.01376516	0.12418721

As noted in the protocol text, if we see one event while the data are still blinded (i.e., 1/180), the best estimate of the minimum overall rate of toxicity in the study is 0.5% but is compatible with a rate of 2.6% with a 90% CI. With 4 events the estimate becomes compatible with a rate of 5%, though the best estimate is still 2.2%. With 5 events the lower bound finally exceeds 1%.

The challenge is that the arm in which the event occurs in them has a minimum rate of toxicity of 1.7% with 90% bounds of 0.001-7.7%. If we wait till we see two events this increases to 3.3% with bounds of 0.006-10%. With three events the lower bound is 1.4%.

So we chose three accumulated events as halting criteria b, for which full SMC should be convened; if all of 3 events had occurred in one arm, the point estimate would be 5% while the lower bound exceeds 1% whether all events were in one arm or not. In neither case at this sample size is the upper bound below 6%; and we have to accept a CI compatible with toxicity rates of 10-12% with this approach. Although these frequency estimates are minimal, we note that the accumulating data seen by the SMC (with denominators lower than 60 or 180) would have a lower degree of confidence than those reported in the tables above.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

18.0 REFERENCES

1. World Health Organization. Global tuberculosis control: epidemiology, strategy, financing. Geneva: World Health Organization, 2009.

2. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946-1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 1999; **3**(10 Suppl 2): S231-79.

3. Fox W. Whither short-course chemotherapy? *Br J Dis* 1981; **75**(4): 331-57.

4. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* 1985; **66**(3): 219-25.

5. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother* 2003; **47**(7): 2118-24.

6. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1980; **121**(6): 939-49.

7. Sirgel FA, Fourie PB, Donald PR, et al. The early bactericidal activities of rifampin and rifapentine in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2005; **172**(1): 128-35.

8. Long MW, Snider DE, Jr., Farer LS. U.S. Public Health Service Cooperative trial of three rifampin-isoniazid regimens in treatment of pulmonary tuberculosis. *Am Rev Respir Dis* 1979; **119**(6): 879-94.

9. Menzies D, Benedetti A, Paydar A, et al. Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. *PLoS Med* 2009; **6**(9): e1000146.

10. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifampin-based treatment: an analytical review. *Clin Infect Dis* 2003; **37**(1): 101-12.

11. Peloquin C. What is the 'right' dose of rifampin? *Int J Tuberc Lung Dis* 2003; **7**(1): 3-5.

12. Mitchison DA. Antimicrobial therapy of tuberculosis: justification for currently recommended treatment regimens. *Semin Respir Crit Care Med* 2004; **25**(3): 307-15.

13. Davies GR, Nuermberger EL. Pharmacokinetics and pharmacodynamics in the development of anti-tuberculosis drugs. *Tuberculosis (Edinb)* 2008; **88 Suppl 1**: S65-74.

14. Mitnick CD, McGee B, Peloquin CA. Tuberculosis pharmacotherapy: strategies to optimize patient care. *Expert Opin Pharmacother* 2009; **10**(3): 381-401.

15. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. *Int J Tuberc Lung Dis* 2000; **4**(9): 796-806.

16. Peloquin CA, Vernon A. Antimycobacterial Agents: Rifamycins for Mycobacterial Infections. In: Yu VL, Edwards G, McKinnon PS, Peloquin C, Morse GD, eds. Antimicrobial Chemotherapy and Vaccines, 2nd edition, Volume II: Antimicrobial Agents. Pittsburgh, PA: ESun Technologies; 2005: 383-402.

17. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 2004; **2**(4): 289-300.

18. Dickinson JM, Jackett PS, Mitchison DA. The effect of pulsed exposures to rifampin on the uptake of uridine- 14 C by Mycobacterium tuberculosis. *Am Rev Respir Dis* 1972; **105**(4): 519-27.

19. Grumbach F. Experimental "in vivo" studies of new antituberculosis drugs: capreomycin, ethambutol, rifampicin. *Tubercle* 1969; **50**: Suppl:12-21.

20. Kradolfer F. Rifampicin, isoniazid, ethambutol, ethionamide, and streptomycin in murine tuberculosis: comparative chemotherapeutic studies. *Antibiot Chemother* 1970; **16**: 352-60.

21. Dickinson JM, Mitchison DA. Experimental models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis. *Am Rev Respir Dis* 1981; **123**(4 Pt 1): 367-71.

22. Verbist L. Rifampicin activity "in vitro" and in established tuberculosis in mice. *Acta Tuberc Pneumol Belg* 1969; **60**(3): 397-412.

23. Burman WJ, Gallicano K, Peloquin C. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. *Clin Pharmacokinet* 2001; **40**(5): 327-41.

24. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrob Agents Chemother* 2006; **50**(4): 1170-7.

25. Namdar R, Ebert S, Peloquin CA. Drugs for TB. Drug Interactions in Infectious Diseases, 2nd Edition. Totowa, NJ: Humana Press; 2000: 191-214.

26. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin : clinical relevance. *Clin Pharmacokinet* 2003; **42**(9): 819-50.

27. Feng HJ, Huang SL, Wang W, Zhou HH. The induction effect of rifampicin on activity of mephenytoin 4'-hydroxylase related to M1 mutation of CYP2C19 and gene dose. *Br J Clin Pharmacol* 1998; **45**(1): 27-9.

28. Branch RA, Herman RJ. Enzyme induction and beta-adrenergic receptor blocking drugs. *Br J Clin Pharmacol* 1984; **17 Suppl 1**: 77S-84S.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

29. Ohnhaus EE, Breckenridge AM, Park BK. Urinary excretion of 6 beta-hydroxycortisol and the time course measurement of enzyme induction in man. *Eur J Clin Pharmacol* 1989; **36**(1): 39-46.

30. Ruslami R, Nijland H, Aarnoutse R, et al. Evaluation of high- versus standard-dose rifampin in Indonesian patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* 2006; **50**(2): 822-3.

31. Decroix G, Kreis B, Sors C, Birembaum J, Le Lirzin M, Canetti G. [Comparison between regimes of rifampicin-isoniazid administered daily and administered twice a week (initial results of a comparative study conducted in 4 medical services of the Parisian region)]. *Rev Tuberc Pneumol (Paris)* 1969; **33**(6): 751-68.

32. Acocella G. Pharmacokinetics and metabolism of rifampin in humans. *Rev Infect Dis* 1983; **5 Suppl 3**: S428-32.

33. Diacon AH, Patientia RF, Venter A, et al. Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears. *Antimicrob Agents Chemother* 2007; **51**(8): 2994-6.

34. Donald PR, Sirgel FA, Venter A, et al. Early bactericidal activity of antituberculosis agents. *Expert Rev Anti Infect Ther* 2003; **1**(1): 141-55.

35. Hafner R, Cohn JA, Wright DJ, et al. Early bactericidal activity of isoniazid in pulmonary tuberculosis. Optimization of methodology. The DATRI 008 Study Group. *Am J Respir Crit Care Med* 1997; **156**(3 Pt 1): 918-23.

36. Kennedy N, Fox R, Kisyombe GM, et al. Early bactericidal and sterilizing activities of ciprofloxacin in pulmonary tuberculosis. *Am Rev Respir Dis* 1993; **148**(6 Pt 1): 1547-51.

37. Sirgel FA, Botha FJ, Parkin DP, et al. The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts: a new method of drug assessment. *J Antimicrob Chemother* 1993; **32**(6): 867-75.

38. Chan SL, Yew WW, Ma WK, et al. The early bactericidal activity of rifabutin measured by sputum viable counts in Hong Kong patients with pulmonary tuberculosis. *Tuber Lung Dis* 1992; **73**(1): 33-8.

39. Johnson JL, Hadad DJ, Boom WH, et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2006; **10**(6): 605-12.

40. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2008; **178**(11): 1180-5.

41. Warren RM, Victor TC, Streicher EM, et al. Patients with active tuberculosis often have different strains in the same sputum specimen. *Am J Respir Crit Care Med* 2004; **169**(5): 610-4.

42. Post FA, Willcox PA, Mathema B, et al. Genetic polymorphism in Mycobacterium tuberculosis isolates from patients with chronic multidrug-resistant tuberculosis. *J Infect Dis* 2004; **190**(1): 99-106.

43. Aldous WK, Pounder JI, Cloud JL, Woods GL. Comparison of six methods of extracting Mycobacterium tuberculosis DNA from processed sputum for testing by quantitative real-time PCR. *J Clin Microbiol* 2005; **43**(5): 2471-3.

44. Furesz S. Chemical and biological properties of rifampicin. *Antibiot Chemother* 1970; **16**: 316-51.

45. Sensi P, Maggi N, Furesz S, Maffii G. Chemical modifications and biological properties of rifamycins. *Antimicrob Agents Chemother (Bethesda)* 1966; **6**: 699-714.

46. Schiatti P, Maggi N, Sensi P, Maffii G. Biliary excretion rate of semisynthetic rifamycins in the rat. *Chemotherapy* 1967; **12**(3): 155-71.

47. Mehta JB, Shantaveerapa H, Byrd RP, Jr., Morton SE, Fountain F, Roy TM. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. *Chest* 2001; **120**(5): 1520-4.

48. Kimerling ME, Phillips P, Patterson P, Hall M, Robinson CA, Dunlap NE. Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. *Chest* 1998; **113**(5): 1178-83.

49. Saukkonen JJ, Cohn DL, Jasmer RM, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. *Am J Respir Crit Care Med* 2006; **174**(8): 935-52.

50. McEvoy GK, Snow EK, Kester L, editors. AHFS Drug Information 2006. Bethesda, MD: American Society of Health-System Pharmacists; 2006.

51. Peloquin CA. Antituberculosis drugs: pharmacokinetics. In: Heifets LB, ed. Drug-Susceptibility in the chemotherapy of Mycobacterial infections. Boca Raton, FL: CRC Press; 1991: 89-122.

52. Decroix G, Kreis B, Sors C, Birenbaum J, Le Lirzin M, Canetti G. [Comparative study of the treatment of pulmonary tuberculosis by a combination of rifampicin and isoniazid administered daily and twice weekly for a year]. *Rev Tuberc Pneumol (Paris)* 1971; **35**(1): 39-54.

53. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* 1991; **99**(2): 465-71.

54. Sarma GR, Immanuel C, Kailasam S, Narayana AS, Venkatesan P. Rifampin-induced release of hydrazine from isoniazid. A possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampin. *Am Rev Respir Dis* 1986; **133**(6): 1072-5.

55. Pande JN, Singh SP, Khilnani GC, Khilnani S, Tandon RK. Risk factors for hepatotoxicity from antituberculosis drugs: a case-control study. *Thorax* 1996; **51**(2): 132-6.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

56. Lee BH, Koh WJ, Choi MS, et al. Inactive hepatitis B surface antigen carrier state and hepatotoxicity during antituberculosis chemotherapy. *Chest* 2005; **127**(4): 1304-11.

57. Kwon YS, Koh WJ, Suh GY, Chung MP, Kim H, Kwon OJ. Hepatitis C virus infection and hepatotoxicity during antituberculosis chemotherapy. *Chest* 2007; **131**(3): 803-8.

58. de Castro L, do Brasil PE, Monteiro TP, Rolla VC. Can hepatitis B virus infection predict tuberculosis treatment liver toxicity? Development of a preliminary prediction rule. *Int J Tuberc Lung Dis* 2010; **14**(3): 332-40.

59. Girling DJ. Adverse reactions to rifampicin in antituberculosis regimens. *J Antimicrob Chemother* 1977; **3**(2): 115-32.

60. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: Treatment of Tuberculosis. *Am J Respir Crit Care Med* 2003; **167**: 603-62.

61. Dutt AK, Moers D, Stead WW. Undesirable side effects of isoniazid and rifampin in largely twice-weekly short-course chemotherapy for tuberculosis. *Am Rev Respir Dis* 1983; **128**(3): 419-24.

62. Poole G, Stradling P, Worlledge S. Potentially serious side effects of high-dose twice-weekly rifampicin. *Br Med J* 1971; **3**(5770): 343-7.

63. Aquinas M, Allan WG, Horsfall PA, et al. Adverse reactions to daily and intermittent rifampicin regimens for pulmonary tuberculosis in Hong Kong. *Br Med J* 1972; **1**(5803): 765-71.

64. Grosset J, Leventis S. Adverse effects of rifampin. *Rev Infect Dis* 1983; **5 Suppl 3**: S440-50.

65. Girling DJ. Adverse effects of antituberculosis drugs. *Drugs* 1982; **23**(1-2): 56-74.

66. Dhar S, Kaur I, Sharma VK, Kumar B. "Flu" syndrome due to rifampin; experience with four cases. *Int J Lepr Other Mycobact Dis* 1995; **63**(1): 92-4.

67. Bertrand A. [Antibiotic treatment of brucellosis]. *Presse Med* 1994; **23**(24): 1128-31.

68. Acocella G, Bertrand A, Beytout J, et al. Comparison of three different regimens in the treatment of acute brucellosis: a multicenter multinational study. *J Antimicrob Chemother* 1989; **23**(3): 433-9.

69. Reichler MR, Allphin AA, Breiman RF, et al. The spread of multiply resistant Streptococcus pneumoniae at a day care center in Ohio. *J Infect Dis* 1992; **166**(6): 1346-53.

70. Drancourt M, Stein A, Argenson JN, Roiron R, Groulier P, Raoult D. Oral treatment of Staphylococcus spp. infected orthopaedic implants with fusidic acid or ofloxacin in combination with rifampicin. *J Antimicrob Chemother* 1997; **39**(2): 235-40.

71. Baty V, Hoen B, Schuhmacher H, et al. Legionella jordanis pneumonia unresponsive to fluoroquinolones in a non-immunocompromised host. *Scand J Infect Dis* 1997; **29**(3): 319-20.

72. Kochar DK, Aseri S, Sharma BV, Bumb RA, Mehta RD, Purohit SK. The role of rifampicin in the management of cutaneous leishmaniasis. *Qjm* 2000; **93**(11): 733-7.

73. Patel AM, McKeon J. Avoidance and management of adverse reactions to antituberculosis drugs. *Drug Saf* 1995; **12**(1): 1-25.

74. Ormerod LP, Horsfield N. Frequency and type of reactions to antituberculosis drugs: observations in routine treatment. *Tuber Lung Dis* 1996; **77**(1): 37-42.

75. Taki H, Ogawa K, Murakami T, Nikai T. Epidemiological survey of hyperuricemia as an adverse reaction to antituberculous therapy with pyrazinamide. *Kekkaku* 2008; **83**(7): 497-501.

76. Zierski M, Bek E. Side-effects of drug regimens used in short course chemotherapy for pulmonary tuberculosis: a controlled clinical study. *Tubercle* 1980; **61**(1): 41-9.

77. Combs DL, O'Brien RJ, Geiter LJ. USPHS tuberculosis short-course chemotherapy trial 21: effectiveness, toxicity, and acceptability. *Ann Intern Med* 1990; **112**(6): 397-406.

78. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *Am J Respir Crit Care Med* 2003; **167**(11): 1472-7.

79. Schaberg T, Rebhan K, Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *Eur Respir J* 1996; **9**(10): 2026-30.

80. Dossing M, Wilcke JT, Askgaard DS, Nybo B. Liver injury during antituberculosis treatment: an 11-year study. *Tuber Lung Dis* 1996; **77**(4): 335-40.

81. Girling DJ. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle* 1978; **59**(1): 13-32.

82. Grange JM, Winstanley PA, Davies PD. Clinically significant drug interactions with antituberculosis agents. *Drug Saf* 1994; **11**(4): 242-51.

83. Yew WW. Clinically significant interactions with drugs used in the treatment of tuberculosis. *Drug Saf* 2002; **25**(2): 111-33.

84. Israili ZH, Rogers CM, el-Attar H. Pharmacokinetics of antituberculosis drugs in patients. *J Clin Pharmacol* 1987; **27**(1): 78-83.

85. Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet* 1984; **9**(6): 511-44.

86. Jain A, Mehta VL, Kulshrestha S. Effect of pyrazinamide on rifampicin kinetics in patients with tuberculosis. *Tuber Lung Dis* 1993; **74**(2): 87-90.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

87. Schluger N, Karunakara U, Lienhardt C, Nyirenda T, Chaisson R. Building clinical trials capacity for tuberculosis drugs in high-burden countries. *PLoS Med* 2007; **4**(11): e302.

88. UN Millennium Project. Chapter Four: Moving Forward to Achieve Millenium Development Goals Target for TB. In: Broekmans J, Caines K, Paluzzi J, eds. Investing in strategies to reduce the global incidence of TB. London: Earthscan; 2005: 67-8.

89. Chee CB, Boudville IC, Chan SP, Zee YK, Wang YT. Patient and disease characteristics, and outcome of treatment defaulters from the Singapore TB control unit--a one-year retrospective survey. *Int J Tuberc Lung Dis* 2000; **4**(6): 496-503.

90. Thomas A, Gopi PG, Santha T, et al. Predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India. *Int J Tuberc Lung Dis* 2005; **9**(5): 556-61.

91. Kim DH, Kim HJ, Park SK, et al. Treatment outcomes and survival based on drug resistance patterns in multidrug-resistant tuberculosis. *Am J Respir Crit Care Med* 2010; **182**(1): 113-9.

92. Donald PR, Sirgel FA, Botha FJ, Seifart HI, Parkin DP, Vandenplas ML, et al. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. *American journal of respiratory and critical care medicine* 1997; **156**(3 pt 1): 895-900.

93. Institute CaLS. Susceptibility testing of *Mycobacteria, Nocardiae*, and other aeorbic *Actinomycetes*: approved standard. Wayne, PA: Clinical and Laboratory Standards Institute, 2003.

94. Davies GR, Khoo SH, Aarons LJ. Optimal sampling strategies for early pharmacodynamic measures in tuberculosis. *J Antimicrob Chemother* 2006; **58**(3): 594-600.

95. Rustomjee R, Lienhardt C, Kanyok T, et al. A Phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2008; **12**(2): 128-38.

96. Davies GR, Brindle R, Khoo SH, Aarons LJ. Use of nonlinear mixed-effects analysis for improved precision of early pharmacodynamic measures in tuberculosis treatment. *Antimicrob Agents Chemother* 2006; **50**(9): 3154-6.

97. Chow S-C, Liu J-P. Design and analysis of clinical trials:concepts and methodologies. 2nd ed. Hoboken, New Jersey: John Wiley; 2004.

98. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. *Antimicrob Agents Chemother* 2007; **51**(11): 3781-8.

99. Cohn ML, Waggoner RF, McClatchy JK. The 7H11 medium for the cultivation of mycobacteria. *Am Rev Respir Dis* 1968; **98**(2): 295-6.

100. Brander E. A simple way of detecting pyrazinamide resistance. *Tubercle* 1972; **53**(2): 128-31.

101. Pinheiro VG, Ramos LM, Monteiro HS, et al. Intestinal permeability and malabsorption of rifampin and isoniazid in active pulmonary tuberculosis. *Braz J Infect Dis* 2006; **10**(6): 374-9.

102. Peloquin CA, Namdar R, Singleton MD, Nix DE. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. *Chest* 1999; **115**(1): 12-8.

103. Weiner M, Burman W, Luo CC, et al. Effects of rifampin and multidrug resistance gene polymorphism on concentrations of moxifloxacin. *Antimicrob Agents Chemother* 2007; **51**(8): 2861-6.

104. Rifapentine (Priftin) [package insert]. Kansas City: Hoechst Marion Roussel, 1998.

105. Baker SG, Freedman LS. A simple method for analyzing data from a randomized trial with a missing binary outcome. *BMC Med Res Methodol* 2003; **3**: 8.

106. Wu MC, Follmann DA. Use of summary measures to adjust for informative missingness in repeated measures data with random effects. *Biometrics* 1999; **55**(1): 75-84.

107. Wilkins JJ, Savic RM, Karlsson MO, et al. Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption. *Antimicrob Agents Chemother* 2008; **52**(6): 2138-48.

108. Sirgel FA, Donald PR, Odhiambo J, et al. A multicentre study of the early bactericidal activity of anti- tuberculosis drugs. *J Antimicrob Chemother* 2000; **45**(6): 859-70.

109. UN Millennium Project. Investing in strategies to reverse the global incidence of TB: Task Force on HIV/AIDS, Malaria, TB, and Access to Essential Medicines, 2005.

110. Diacon AH, Pym A, Grobusch M, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 2009; **360**(23): 2397-405.

111. Stop TB Partnership. The Global Plan to Stop TB 2006-2015: Actions for life towards a world free of tuberculosis. Geneva: World Health Organization, 2006.

112. Barry CE, 3rd, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 2009.

113. Mukamolova GV, Turapov O, Malkin J, Woltmann G, Barer MR. Resuscitation Promoting Factors Reveal an Occult Population of Tubercle Bacilli in Sputum. *Am J Respir Crit Care Med* 2009.

114. Garton NJ, Waddell SJ, Sherratt AL, et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. *PLoS Med* 2008; **5**(4): e75.

115. Jindani A, Dore CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *Am J Respir Crit Care Med* 2003; **167**(10): 1348-54.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

116. Phillips P. Surrogate markers for poor outcome treatment for tuberculosis: results from extensive multi-trial analysis. 39th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease; 2008 October 16-20, 2008; Paris, France: International Journal of Tuberculosis and Lung Disease Supplement; 2008. p. s146.

117. Maggi N, Pasqualucci CR, Ballotta R, Sensi P. Rifampicin: a new orally active rifamycin. *Chemotherapy* 1966; **11**(5): 285-92.

118. Lee CN, Heifets LB. Determination of minimal inhibitory concentrations of antituberculosis drugs by radiometric and conventional methods. *Am Rev Respir Dis* 1987; **136**(2): 349-52.

119. Hobby GL. Summation of experimental studies on the action of rifampin. *Chest* 1972; **61**(6): 550-4.

120. Trnka L. Rifampicin. In: Bartmann K, ed. Handbook of Experimental Pharmacology: Antituberculosis Drugs. Berlin: Springer-Verlag: 205-32.

121. Nitti V. Antituberculosis activity of rifampin. Report of studies performed and in progress (1966-1971). *Chest* 1972; **61**(6): 589-98.

122. Boman G, Ringberger VA. Binding of Rifampicin by Human Plasma Proteins. *European Journal of Clinical Pharmacology* 1974; **7**: 369-73.

123. Rodriguez JC, Cebrian L, Ruiz M, Lopez M, Royo G. Mutant prevention concentration of isoniazid, rifampicin and rifabutin against Mycobacterium tuberculosis. *Chemotherapy* 2005; **51**(2-3): 76-9.

124. Verbist L, Gyselen A. Antituberculous Activity of Rifampin In Vitro and In Vivo and the Concentrations Attained in Human Blood. *American Review of Respiratory Disease* 1968; **98**(6): 923-32.

125. Dhillon J, Mitchison DA. Activity and penetration of antituberculosis drugs in mouse peritoneal macrophages infected with Mycobacterium microti OV254. *Antimicrob Agents Chemother* 1989; **33**(8): 1255-9.

126. Chanwong S, Maneekarn N, Makonkawkeyoon L, Makonkawkeyoon S. Intracellular growth and drug susceptibility of Mycobacterium tuberculosis in macrophages. *Tuberculosis (Edinb)* 2007; **87**(2): 130-3.

127. Crowle AJ, Elkins N, May MH. Effectiveness of ofloxacin against Mycobacterium tuberculosis and Mycobacterium avium, and rifampin against M. tuberculosis in cultured human macrophages. *Am Rev Respir Dis* 1988; **137**(5): 1141-6.

128. Dickinson JM, Mitchison DA. Experimental investigations of bacteriological mechanisms in short course chemotherapy. *Bull Int Union Tuberc* 1976; **51**(1): 79-86.

129. Grosset J. The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. *Bull Int Union Tuberc* 1978; **53**(1): 5-12.

130. Chen J, Raymond K. Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor. *Ann Clin Microbiol Antimicrob* 2006; **5**: 3.

131. Acocella G, Nonis A, Gialdroni-Grassi G, Grassi C. Comparative bioavailability of isoniazid, rifampin, and pyrazinamide administered in free combination and in a fixed triple formulation designed for daily use in antituberculosis chemotherapy. I. Single-dose study. *Am Rev Respir Dis* 1988; **138**(4): 882-5.

132. Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J Pharmacol Exp Ther* 2001; **299**(3): 849-57.

133. Langdon G, Wilkins J, McFadyen L, McIlleron H, Smith P, Simonsson US. Population pharmacokinetics of rifapentine and its primary desacetyl metabolite in South African tuberculosis patients. *Antimicrob Agents Chemother* 2005; **49**(11): 4429-36.

134. McIlleron H, Wash P, Burger A, Folb P, Smith P. Widespread distribution of a single drug rifampicin formulation of inferior bioavailability in South Africa. *Int J Tuberc Lung Dis* 2002; **6**(4): 356-61.

135. Verbist L. Pharmacological Study of Rifampicin after Repeated High Dosage during Intermittent Combined Therapy. *Respiration* 1971; **28**(supplement): 7-16.

136. van Crevel R, Alisjahbana B, de Lange WC, et al. Low plasma concentrations of rifampicin in tuberculosis patients in Indonesia. *Int J Tuberc Lung Dis* 2002; **6**(6): 497-502.

137. Goutelle S, Bourguignon L, Maire PH, Van Guilder M, Conte JE, Jr., Jelliffe RW. Population modeling and Monte Carlo simulation study of the pharmacokinetics and antituberculosis pharmacodynamics of rifampin in lungs. *Antimicrob Agents Chemother* 2009; **53**(7): 2974-81.

138. Verbist L, Rollier F. Pharmacological study of rifampicin after repeated high dosage during intermittent combined therapy. II. Bilirubin levels and other biochemical determinations. *Respiration* 1971; **28**: Suppl:17-28.

139. Kreis B, Pretet S, Birenbaum J, et al. Two Three-Month Treatment Regimens for Pulmonary TB. *Bull Int Un Tuberc* 1976; **51**: 71-5.

140. East African/British Medical Research Council. Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. *Lancet* 1972; **1**(7760): 1079-85.

141. A comparative study of daily followed by twice or once weekly regimens of ethambutol and rifampicin in retreatment of patients with pulmonary tuberculosis. The results at 1 year. A cooperative tuberculosis chemotherapy study in Poland. *Tubercle* 1975; **56**(1): 1-26.

142. A Cooperative Tuberculosis Chemotherapy Study in Poland from the National Research Institute for Tuberculosis PSDoTaRD. A Comparative Study of Daily Followed by Twice- or Once-Weekly Regimens

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

of Ethambutol and Rifampicin in the Retreatment of Patients with Pulmonary Tuberculosis: Second Report. *Tubercle* 1976; **57**: 105-13.

143. Hong Kong Tuberculosis Treatment Services, Brompton Hospital, British Medical Research Council. A controlled trial of daily and intermittent rifampicin plus ethambutol in the retreatment of patients with pulmonary tuberculosis: results up to 30 months. *Tubercle* 1975; **56**(3): 179-89.

144. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. *Antimicrob Agents Chemother* 2007; **51**(7): 2546-51.

145. Agarwal SK, Roy, D.C., Pal, D. Once a week short-term tuberculosis therapy. *Asian Medical Journal* 1983; **26**(6): 390-6.

146. Weiner M PC, Burman W, Luo CC, Engle M, Prihoda T, Mac Kenzie W, Bliven E, Johnson J, Vernon A for the Tuberculosis Trials Consortium. The effects of tuberculosis infection, race, and human gene SLCO1B1 polymorphisms on rifampin concentrations. 2nd International Workshop on Clinical Pharmacology of Tuberculosis Drugs. San Francisco, CA; 2009.

147. Felgenhauer F, Lagler F. Experimental animal investigations with rifampicin on the question of its influence on the vestibular system and its period of retention in the perilymph of the inner ear. *Antibiot Chemother* 1970; **16**: 361-8.

148. Kluyskens P. [Experimental research on otovestibular toxicity of rifomycin]. *Acta Tuberc Pneumol Belg* 1969; **60**(3): 323-8.

149. Hugues FC, Marche C, Marche J. [Hepato-biliary effect of the rifomycin-prothionamid combination. I. Histological study on the rat]. *Therapie* 1970; **25**(1): 131-6.

150. Martinez E, Collazos J, Mayo J. Hypersensitivity reactions to rifampin. Pathogenetic mechanisms, clinical manifestations, management strategies, and review of the anaphylactic-like reactions. *Medicine (Baltimore)* 1999; **78**(6): 361-9.

151. Humber DP, Nsanzumuhire H, Aluoch JA, et al. Controlled double-blind study of the effect of rifampin on humoral and cellular immune responses in patients with pulmonary tuberculosis and in tuberculosis contacts. *Am Rev Respir Dis* 1980; **122**(3): 425-36.

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

153. Halsey NA, Coberly JS, Desormeaux J, et al. Randomised trial of isoniazid versus rifampicin and pyrazinamide for prevention of tuberculosis in HIV-1 infection. *Lancet* 1998; **351**(9105): 786-92.

154. Mwinga A, Hosp M, Godfrey-Faussett P, et al. Twice weekly tuberculosis preventive therapy in HIV infection in Zambia. *AIDS* 1998; **12**(18): 2447-57.

155. Jasmer RM, Daley CL. Rifampin and pyrazinamide for treatment of latent tuberculosis infection: is it safe? *Am J Respir Crit Care Med* 2003; **167**(6): 809-10.

156. Stout JE, Engemann JJ, Cheng AC, Fortenberry ER, Hamilton CD. Safety of 2 months of rifampin and pyrazinamide for treatment of latent tuberculosis. *Am J Respir Crit Care Med* 2003; **167**(6): 824-7.

157. Jasmer RM, Saukkonen JJ, Blumberg HM, et al. Short-course rifampin and pyrazinamide compared with isoniazid for latent tuberculosis infection: a multicenter clinical trial. *Ann Intern Med* 2002; **137**(8): 640-7.

158. Bock NN, Rogers T, Tapia JR, Herron GD, DeVoe B, Geiter LJ. Acceptability of short-course rifampin and pyrazinamide treatment of latent tuberculosis infection among jail inmates. *Chest* 2001; **119**(3): 833-7.

159. McNeill L, Allen M, Estrada C, Cook P. Pyrazinamide and rifampin vs isoniazid for the treatment of latent tuberculosis: improved completion rates but more hepatotoxicity. *Chest* 2003; **123**(1): 102-6.

160. Burman WJ, Peloquin CA. Isoniazid. In: Yu VL, Edwards G, McKinnon PS, Peloquin C, GD M, eds. Antimicrobial Chemotherapy and Vaccines, 2nd edition, Volume II: Antimicrobial Agents

Esun Technologies, LLC; 2005: 539-50.

161. Weiner M, Bock N, Peloquin CA, et al. Pharmacokinetics of rifapentine at 600, 900, and 1,200 mg during once-weekly tuberculosis therapy. *Am J Respir Crit Care Med* 2004; **169**(11): 1191-7.

162. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. *Int J Tuberc Lung Dis* 2003; **7**(1): 6-21.

163. Heifets L. Pyrazinamide. In: Yu VL., Edwards G., McKinnon PS., Peloquin C., Morse GD., eds. Antimicrobial Chemotherapy and Vaccines, 2nd edition, Volume II: Antimicrobial Agents: Esun Technologies, LLC; 2005: 559-69.

164. Lewis ML. Ethambutol. In: Yu VL, Edwards G, McKinnon PS, Peloquin C, GD. M, eds. Antimicrobial Chemotherapy and Vaccines, 2nd Edition, Volume II: Antimicrobial Agents: Esun Technologies, LLC; 2005: 523-32.

165. World Health Organization Public Assessment Reports. Part 4. Summary of Product Characteristics. Isoniazid Tablets 300mg (Micro Labs Limited) TB174

2011.

166. World Health Organization Public Assessment Reports. Part 4. Summary of Product Characteristics. Pyrazinamide Tablets 400mg (Macleods) TB159. 2012.

AJRCCM Articles in Press. Published on 29-June-2018 as 10.1164/rccm.201712-2524OC

167. World Health Organization Public Assessment Reports. Part 4. Summary of Product Characteristics. Ethambutol Tablets 400mg (Macleods) TB134. Version 1.0. 2012.