Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: A randomized, open-label, controlled phase 3 clinical trial

Final and original study protocol and statistical analysis plans

Consortium Identifiers:

- Tuberculosis Trials Consortium Study 31
- AIDS Clinical Trials Group A5349

Table of contents:

*v1.1 (24 Nov 2014) was the first version of the study protocol that was implemented, amended from v1.0 after comments from IRBs prior to study start.

TITLE

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Funding Agencies:
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Study Chairs:
Payam Nahid, M.D., M.P.H.
Susan Dorman, M.D.

Version Number: 2.0

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Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR, and applicable site-specific regulatory requirements.

Table of Contents

Protocol Summary

Title: Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: a randomized, open-label, controlled, phase 3 clinical trial

Hypotheses:

A) Seventeen (17) week rifapentine-based regimen

In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine (P), isoniazid (H), pyrazinamide (Z) and ethambutol (E) followed by nine weeks of rifapentine plus isoniazid, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin (R), isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

B) Seventeen (17) week rifapentine- plus moxifloxacin-containing regimen

In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide and moxifloxacin (M), followed by nine weeks of rifapentine, isoniazid, and moxifloxacin, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

Phase: 3

Design: This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.

Population: Patients with newly diagnosed, previously untreated pulmonary tuberculosis.

Number of Sites: Multiple international sites, primarily sites of the Tuberculosis Trials Consortium and the AIDS Clinical Trials Group.

Study Duration: Duration per participant is approximately 18 months.

Description of Agent or Intervention: After written informed consent, participants will be randomly assigned to receive one of the following oral regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

Objectives:

Primary:

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

Secondary:

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and assess biospecimens from consenting participants for the purpose of research on discovery and validation of TB biomarkers

- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, culture status at completion of eight weeks of treatment, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among patients with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine

Endpoints:

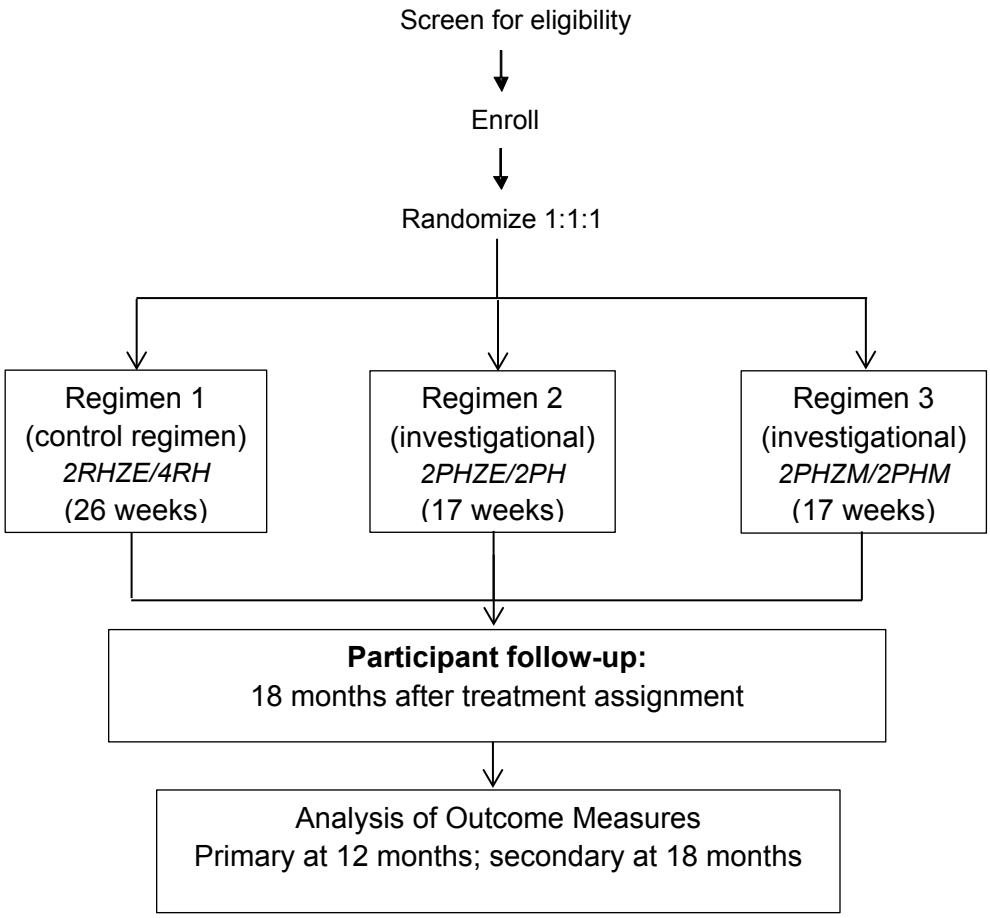
Primary Endpoints:

- Efficacy: TB disease-free survival at twelve months after study treatment assignment.
- Safety: Proportion of participants with grade 3 or higher adverse events during study drug treatment

Secondary Endpoints:

- TB disease-free survival at eighteen months after study treatment assignment
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection
- Proportion of participants who are culture negative at completion of eight weeks of treatment (solid and liquid media considered separately)
- Sensitivity analyses assuming all participants classified as ‘not assessable’ have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

Schematic of Study Design:



1 KEY ROLES

Funding Agencies:
U.S. Centers for Disease Control and Prevention through the Tuberculosis Trials Consortium;
U.S. National Institute of Allergy and Infectious Diseases of the National Institutes of Health through the AIDS Clinical Trials Group

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Protocol Chairs:
Payam Nahid, M.D., M.P.H.
University of California, San Francisco
1001 Potrero Ave, 5K1
San Francisco, CA 94110
Phone: 415-206-5464
Email: pnahid@ucsf.edu

Susan E. Dorman, M.D.
Johns Hopkins University School of Medicine
1550 Orleans Street, CRB2, 1M-12
Baltimore, Maryland, USA 21231
Phone 410-502-2717
Email: dsusan1@jhmi.edu

Project Officer:
Stefan Goldberg, M.D.
US Centers for Disease Control and Prevention
1600 Clifton Road, MS E-10
Atlanta, GA, USA 30333
Phone: 404-639-5339
Email: ssg3@cdc.gov

Central Study Clinician:
TB clinician(s), TBD

Protocol Team

Name	Institution
Janet Andersen	Harvard School of Public Health, Boston, Massachusetts, USA
Richard Chaisson	Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Kwok-Chiu Chang	TB and Chest Service of Hong Kong, China
Michael Chen	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Mark Cotton	Stellenbosch University, Cape Town, South Africa
Dalene von Delft	Community Research Advisory Group, Cape Town, South Africa
Kelly Dooley	Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Melissa Engle	University of Texas Health Science Center, San Antonio, Texas, USA
Pei-Jean Feng	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Courtney Fletcher	University of Nebraska Medical Center, Omaha, Nebraska, USA
Phan Ha	National TB Program, Hanoi, Vietnam
Charles M Heilig	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Daniel Johnson	Division of AIDS, National Institutes of Health, Bethesda, Maryland, USA
John L. Johnson	Case Western Reserve University, Cleveland, Ohio, USA
Marilyn Maroni	Sanofi, Paris, France
Cynthia Merrifield	University of California, San Francisco, San Francisco, California, USA
Jose M. Miro	Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona, Spain
Sachiko Miyahara	Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
Nguyen Viet Nhung	National TB Program, Hanoi, Vietnam
April Pettit	Vanderbilt University, Nashville, Tennessee, USA
Anthony Podany	University of Nebraska Medical Center, Omaha, Nebraska, USA
Kathleen Robergeau	Westat, Inc., Rockville, Maryland, USA
Wadzanai Samaneka	Parirenyatwa Clinical Research Site, Harare, Zimbabwe
Erin Sizemore	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Susan Swindells	University of Nebraska Medical Center, Omaha, Nebraska, USA
Andrew Vernon	Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Marc Weiner	Audie L. Murphy Veterans Affairs Medical Center / University of Texas Health Science Center, San Antonio, Texas, USA
Lisa Wolf	Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Suria Yesmin	Social & Scientific Systems, Inc., Silver Spring, Maryland, USA

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Tuberculosis as a global health problem

Tuberculosis (TB) is one of the most important global health problems. According to recent estimates from the World Health Organization (WHO), 8.6 million new cases and 1.3 million deaths from TB occurred in 2012 (World Health Organization 2013). The vast majority of TB cases and TB deaths are in developing countries. The spread of HIV has fueled the TB epidemic, and TB is the leading cause of death among patients infected with HIV (Corbett et al., 2003). TB predominantly affects young adults in their most productive years of life and has substantial impact on economic development.

Need for new treatment regimens for tuberculosis

Although effective therapy for drug susceptible *Mycobacterium tuberculosis* is available, TB continues to cause significant morbidity and mortality worldwide, and rates of multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB cases are on the rise. A major obstacle to the control of TB is poor adherence with lengthy (at minimum 6 months) and complicated treatment regimens. Incomplete TB treatment can lead to increased morbidity and mortality, prolonged infectiousness and transmission, and the development of drug resistance. The use of directly observed therapy (DOT) can improve patient adherence and reduce the emergence of resistant microorganisms, but is logistically difficult and expensive to implement (McDonald et al., 1982). The development of new treatment strategies with more potent antimycobacterial activity could lead to shorter and better tolerated regimens. A TB treatment regimen that allowed a decrease in treatment duration would potentially have important public health implications by facilitating DOT, increasing cure rates, potentially reducing transmission and preventing emergence of MDR TB. It is estimated that improved regimens that shorten treatment duration for drug-susceptible strains and are efficacious against resistant strains could reduce the incidence of TB by up to 27% by 2050 and reduce deaths by 25% from current global numbers of incident cases and deaths per year (Abu-Raddad et al, 2009).

Current standard treatment for pulmonary tuberculosis

Modern short course treatment for pulmonary tuberculosis is comprised of two treatment phases. The intensive phase is the initial 8 weeks of treatment, and typically is comprised of isoniazid, rifampin, pyrazinamide, and ethambutol. Continuation phase follows intensive phase, and continuation phase typically is comprised of isoniazid plus rifampin for an additional 18 weeks, to complete a total of 26 weeks (6 months) of treatment.

Rifamycins in tuberculosis treatment

Rifamycins are the key drugs in modern short-course TB chemotherapy of 6 months duration. Rifamycins, including rifampin and rifapentine, have concentration-dependent activity against *M. tuberculosis.* Rifamycins are considered critical for sterilization, that is, prevention of relapse after cessation of TB treatment. For rifapentine, the minimum inhibitory concentration (MIC)50 and MIC90 are one- to two-fold dilutions lower than those of rifampin (for the 7H10 agar system, rifapentine's MIC50 and MIC90 are 0.125 and 0.25 mg/L, compared with 0.5 and 1.0 mg/L for rifampin) (Bemer-Melchior et al., 2000). In addition, rifapentine's half-life (t1/2) is five times longer than that of rifampin (14-18 hours vs. 2-5 hours).

Preclinical studies of rifapentine

Murine model of tuberculosis treatment

The murine model of TB has been used for more than 50 years for the development and evaluation of new antituberculosis drugs and regimens (Veziris et al., 2005). Importantly, the mouse model of TB treatment has been shown to recapitulate human TB treatment with regard to treatment-shortening effects of rifampin and pyrazinamide. In the mouse model, the standard 6-month rifampin plus isoniazid plus pyrazinamide (RHZ)-based regimen cures mice in 6 months, followed by relapse rates of 0-10%. On the basis of its recapitulation of outcomes in humans, the murine TB treatment model is the reference standard against which new treatments are compared.

Preclinical studies of tuberculosis treatment regimens containing rifapentine

Pre-clinical studies suggest that improved antimycobacterial activity can be achieved with rifamycin exposure greater than that of the current standard regimen in which rifampin is used at a dose of 10 mg/kg/dose (almost always given as 600 mg) once daily. Increased rifamycin exposure can be achieved by using rifapentine. Of note, the pharmacokinetics of rifapentine have been shown to be similar in mice and in humans (Rosenthal et al., 2005). In the murine model of TB treatment, once-daily rifapentine administered during intensive phase has very potent antimycobacterial activity that results in durable cure after only 3 to 4 months of total treatment (Rosenthal et al., 2008). After aerosol infection, mice achieved a bacillary burden of 7.21 $\log_{10}$ cfu per lung. Treatment with a standard regimen of daily rifampin (10 mg/kg) plus isoniazid and pyrazinamide resulted in a decrease in bacillary burden of approximately 3 logs at completion of 4 weeks of treatment. However, treatment with a regimen of daily rifapentine (10 mg/kg) plus isoniazid and pyrazinamide was significantly more active at 4 weeks (mean lung cfu counts that were 1.00 log10 cfu lower than those for the standard regimen, $p<0.001$) and at 8 weeks (mean lung cfu counts that were >2 log10 cfu lower than those for the standard regimen, $p<0.001$). Furthermore, after 12 weeks of treatment, 100% (15/15) of mice treated with the standard regimen had bacteriological relapse, compared to 0/15 mice treated with the rifapentine regimen. In fact, the rifapentine regimen resulted in cure of 15/15 (100%) of mice after treatment for only 10 weeks.

Thus, murine studies indicate that rifapentine administered daily during combination intensive phase treatment has potent antimycobacterial activity that is associated with ability to achieve durable cure without relapse after about 3 months of total treatment.

Clinical trials of daily rifapentine

Phase I

Dooley and colleagues conducted a phase I dose-escalation study among healthy adult volunteers to determine the safety and pharmacokinetics of escalating daily doses of rifapentine (Dooley et al, 2012). Participants received 5, 10, 15, or 20 mg/kg/dose rifapentine given once daily for 15 consecutive days; 20 mg/kg/dose was the pre-specified maximum dose; a cohort of additional participants received rifampin 10 mg/kg/dose. Of note, this study used strict weight-based dosing, such that the average rifapentine dose administered in the 20 mg/kg/dose cohort was 1650 mg daily. A total of 33 participants received study drugs. There were no grade 2 or higher clinical adverse events. Dose-limiting toxicities were observed in three participants, one each in the rifampin (grade 3 neutropenia), rifapentine 10 mg/kg (grade 3 serum liver transaminase elevation), and rifapentine 15 mg/kg (grade 3 lymphopenia) cohorts. In this study, the safety profile of rifapentine was similar to that of rifampin 10 mg/kg/dose, and it was concluded that rifapentine administered daily was tolerated and safe at doses as high as 20 mg/kg/dose. From a PK perspective, increases in rifapentine dosage resulted in less-than-dose proportional increases in single and multiple dose maximal concentrations.

Phase 2

The TBTC recently completed two phase 2 studies to assess the antimicrobial activity, safety, and tolerability of daily rifapentine administered with isoniazid, PZA, and ethambutol during the first eight weeks of pulmonary TB treatment. In TBTC Study 29, 531 adults with sputum smear positive pulmonary TB were randomized to receive rifapentine approximately 10 mg/kg/dose or rifampin 10 mg/kg/dose administered 5 days per week for 8 weeks (intensive phase) with isoniazid, PZA, and ethambutol; study drugs were administered on an empty stomach (Dorman et al, 2012). The co-primary endpoints were negative sputum cultures on liquid and on solid media at the end of intensive phase; safety and tolerability were also assessed. This study demonstrated no significant difference between regimens in antimicrobial activity based on the surrogate marker of culture status at completion of intensive phase (culture conversion on solid media 83.3% vs. 86.4% for the rifampin vs. rifapentine groups; and conversion in liquid media 65.1% vs. 67.9% in the rifampin vs. rifapentine groups). The rifapentine regimen was well tolerated based on similar proportions of participants discontinuing assigned treatment overall (15.7% in the rifampin group vs. 14.5% in the rifapentine group) or due to toxicity (1.2% in the rifampin group vs. 1.5% in the rifapentine group). There were no differences, by treatment group, in proportions of participants with a serious adverse event related to study treatment (0.4% in the rifampin group vs. 1.1% in the rifapentine group), or by type or severity of adverse events. Hepatitis occurred in 2.8% of rifampin group participants vs. 4.0% of rifapentine group participants (p=0.48). The investigators concluded that the rifapentine regimen administered on an empty stomach 5 days/week for 8 weeks was safe and well-tolerated but not significantly more active than the conventional rifampin regimen.

The TBTC subsequently conducted a randomized, multicenter, dose-ranging study to determine the optimal dose of daily rifapentine during the first 8 weeks of pulmonary TB treatment. In TBTC Study 29X, 334 adults with sputum smear positive pulmonary TB were randomized to receive rifampin (approximately 10 mg/kg/dose) or rifapentine (approximately 10, 15, or 20 mg/kg/dose, maximum dose 1500 mg) administered with a high fat meal once daily for 8 weeks, in addition to isoniazid, PZA, and ethambutol. Rifapentine was well-tolerated across all treatment arms based on a pre-specified definition and also based on comparison with the rifampin group. Percentages of participants discontinuing assigned treatment were: rifampin 11/85 (12.9%; upper bound of 90% one-sided CI 19.0); rifapentine 10 mg/kg 5/87 (5.7%; 10.5); rifapentine 15 mg/kg 5/81 (6.2%; 11.3); and rifapentine 20 mg/kg 9/81 (11.1%; 17.1). There were two deaths – one in the rifapentine 15 mg/kg group due to hematemesis, and one sudden death in the rifapentine 20 mg/kg group in a 61 year old male with untreated hypertension and diabetes mellitus and a strong family history of cardiac disease and sudden death. There were no differences between treatment groups in the percentages of participants with a serious adverse event associated with study treatment, or by type or severity of adverse events. Serious adverse events attributed to study treatment were as follows, by treatment assignment: two events among 85 participants in the rifampin group (one hepatitis, one drug allergy); one event among 87 in the rifapentine 10 mg/kg group (leukocytosis); no events among 81 participants in the rifapentine 15 mg/kg group; one event among 81 participants in the rifapentine 20 mg/kg group (hepatitis). With respect to antimicrobial activity (efficacy), the rifapentine regimens were substantially more active than the standard rifampin regimen based on week 8 (end of intensive phase) culture status (Table 1) as well as time to stable culture conversion.

Antimicrobial activity was associated with rifapentine exposure (area under the concentration time curve [AUC]) (Table 1B). The higher rifapentine exposures were associated with very high rates of sputum sterilization at two months, a very good indicator of overall efficacy of an anti-tuberculosis regimen. Findings were consistent with a steep exposure-response relationship. Pharmacodynamic models were used to further elucidate the relationships between antimycobacterial activity and assigned rifapentine treatment arm, administered rifapentine dose, and rifapentine drug exposure. For efficacy outcomes of time to stable culture conversion in solid media and time to stable culture conversion in liquid media, there was a significant association with rifapentine AUC (p=0.0002 for solid media and p=0.001 for liquid

media) but not for assigned rifapentine mg/kg group (p=0.6 for solid media and p=0.36 for liquid media) or for administered rifapentine dose in mg (p=0.17 for solid media and p=0.17 for liquid media). For rifapentine, the exposure-response relationship was best described by a sigmoidal Emax function (Figure 1), with the greatest change in effect per change in exposure occurring between exposures of approximately 200 mcg*h/L and 550 mcg*h/L, with a plateau in efficacy at higher exposures. In addition to identifying a target AUC of approximately 500 to 600 mcg*h/L, pharmacokinetic studies yielded additional information pertinent for rifapentine dosing. Specifically, the relationship between participant body weight and rifapentine clearance was examined, and clearance was not significantly affected by body weight, thereby supporting 'flat' dosing of rifapentine (i.e. rifapentine dose is not adjusted for body weight) (Figure 2). In addition, pharmacokinetic/pharmacodynamic modeling also predicted that a rifapentine dose of 1200 mg without food would yield an AUC of approximately the same as that of a rifapentine dose of 900 mg with a very high fat meal. Given that target rifapentine AUC lies somewhere between that achieved with a very high fat meal and rifapentine dose of 900 to 1200 mg, the strategy proposed in the current phase 3 trial is a rifapentine dose of 1200 mg with a modest food requirement, with the rationale that a very high fat meal is poorly feasible under phase 3 trial or routine TB care conditions whereas a more general recommendation of dosing with food is likely to be broadly feasible.

Table 1A. Percentages of participants with negative cultures at completion of intensive phase treatment, by treatment assignment, for the modified intention-to-treat analysis group, S29X

	Rifampin	**Rifapentine 10 mg/kg**	**Rifapentine 15 mg/kg**	**Rifapentine 20 mg/kg**
Solid culture medium				
% (n/n) with negative cultures	81.3 (52/64)	92.5 (62/67)	89.4 (59/66)	94.7 (54/57)
% difference vs. Rifampin		11.3	8.1	13.5
(95% CI)		(-1.7, 24.3)	(-5.5, 21.8)	(0.6, 26.3)
p-value		0.10	0.29	0.05
Liquid culture medium				
% (n/n) with negative cultures	56.3 (36/64)	74.6 (50/67)	69.7 (46/66)	82.5 (47/57)
% difference vs. Rifampin		18.4	13.4	26.2
(95% CI)		(0.8, 35.9)	(-4.5, 31.4)	(8.9, 43.5)
p-value		0.04	0.16	<0.01

Table 1B. Percentages of participants with negative cultures at completion of intensive phase treatment, by rifapentine area under the concentration-time curve tertile, for the modified intention-to-treat analysis group, S29X

	Rifampin	**Rifapentine AUC ≤ 323 mcg*h/L**	**Rifapentine AUC 324 to 513 mcg*h/L**	**Rifapentine AUC > 513 mcg*h/L**
Solid culture medium				
% (n/n) with negative cultures	81.3 (52/64)	83.9 (52/62)	100.0 (63/63)	92.3 (60/65)
% difference vs. Rifampin		2.6	18.8	11.1
(95% CI)		(-12.2, 17.4)	(7.6, 29.9)	(-2.0, 24.2)
p-value		0.88	<0.01	0.11
Liquid culture medium				
% (n/n) with negative cultures	56.3 (36/64)	54.8 (34/62)	90.5 (57/63)	80.0 (52/65)
% difference vs. Rifampin		-1.4	34.2	23.8
(95% CI)		(-20.4, 17.5)	(18.5, 50.0)	(6.6, 40.9)
p-value		1.00	<0.01	<0.01

Figure 1. Exposure-response relationship for rifapentine

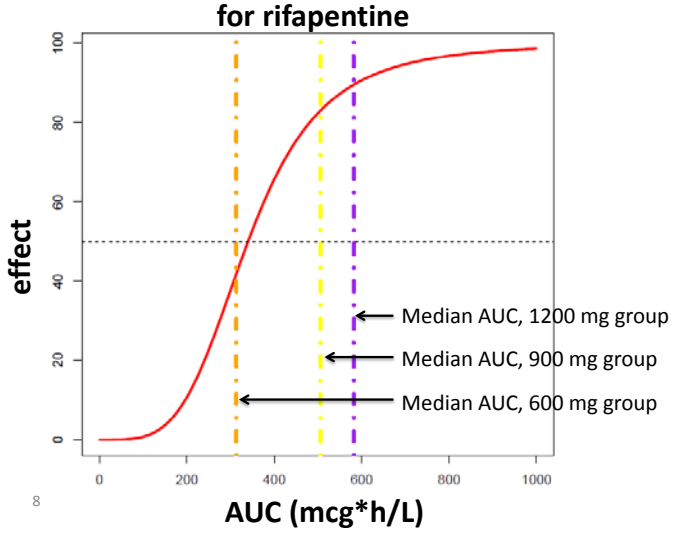
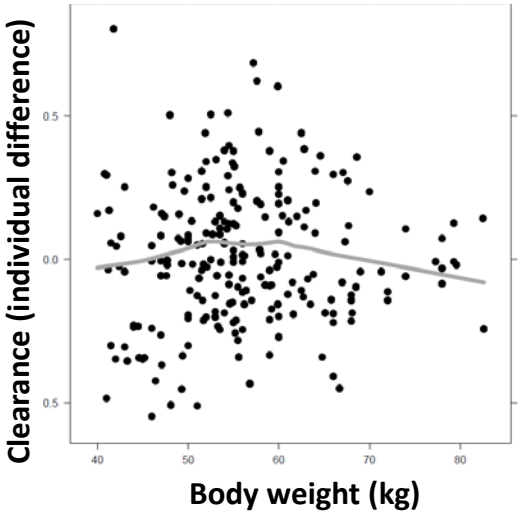


Figure 2. Rifapentine clearance is not meaningfully affected by body weight



Moxifloxacin for TB treatment

Moxifloxacin is a fluoroquinolone with potent activity against *M. tuberculosis* in vitro and in animal models, including sterilizing activity in animal models. In animal models, moxifloxacin's potent activity is partly explained by the fact that it accumulates in granulomas and pulmonary lesions at higher concentrations than found in plasma and lung tissue (Kjellson et al., 2012; Prideaux et al., 2011). In humans, data on the long-term use of moxifloxacin have shown that it has an excellent safety profile. Three phase 2 TB clinical trials have shown that substitution of moxifloxacin for ethambutol during the intensive phase of pulmonary TB treatment increases the antimicrobial activity of the regimen, as assessed using surrogate markers (Burman et al., 2006; Rustomjee et al., 2008; Conde et al., 2009). A recently completed phase 3 trial found that treatment with a weekly regimen of rifapentine and moxifloxacin during the continuation phase of therapy (Rifaquin 6 month regimen, Jindani, 2014) was non-inferior to daily isoniazid plus rifampin; the efficacy of rifapentine plus moxifloxacin in this trial is significant because once-weekly rifapentine with isoniazid (instead of moxifloxacin) is associated with higher rates of relapse and treatment failure (Vernon et al., 1999). Two phase 3 treatment shortening studies using fluoroquinolone-based 4-month regimens administered daily have been completed recently. The Oflotub Trial was an open-label, Phase 3 multicenter trial evaluating the efficacy and safety of a 4-month gatifloxacin (G) containing regimen compared to the standard 6-month HRZE regimen (Merle et al, 2014). The Oflotub 4 month regimen consisted of a 2 month intensive phase of GHRZ, followed by a 2 month continuation phase of GRH (2GRHZ/2GRH) versus a control arm of 2ERHZ/4RH, administered 6 days per week. The investigational 4 month regimen failed to achieve non-inferiority at a 6% margin: in a modified intention-to-treat analysis unfavorable outcomes at 24 months from end-of-treatment had occurred in 21.0% (146/694) in the gatifloxacin-containing arm vs. 17.2% (114/662) in the control arm (difference +3.5%, 95% CI -0.7% to +7.7%). The REMox phase 3 study was a randomized placebo-controlled double-blind trial comparing two treatment shortening regimens, namely 2MHRZ/2MHR and 2EMRZ/2MR, with the standard control regimen 2EHRZ/4HR (Gillespie et al 2014). The primary end point was treatment failure or relapse within 18 months after randomization. In the REMox study, neither of the investigational moxifloxacin-containing regimens was shown to be non-inferior to the control. Specifically, of the 1931

patients who underwent randomization, in the per-protocol analysis, a favorable outcome was reported in fewer patients in the 2MHRZ/2MHR group (85%) and the 2EMRZ/2MR group (80%) than in the control group (2EHRZ/4HR; 92%). A difference favoring the control group of 6.1 percentage points (97.5% confidence interval [CI], 1.7 to 10.5) was noted comparing to the 2MHRZ/2MHR group, and a difference favoring the control group of 11.4 percentage points (97.5% CI, 6.7 to 16.1) was noted when comparing to the 2EMRZ/2MR group. Overall the 2MHRZ/2MHR group performed slightly better than the 2EMRZ/2MR group, and achieved non-inferiority in certain sub-groups (e.g. females). In the REMox study the two moxifloxacin-containing regimens produced a more rapid initial decline in bacterial load as compared to the control group. As in the Oflotub Trial, however, overall the experimental moxifloxacin single-substitution regimen was also not shown to be non-inferior to the control. The fluoroquinolone-containing regimens were safe and well-tolerated in both the Oflotub (gatifloxacin) and the REMox (moxifloxacin) studies. Overall, pre-clinical and clinical studies have shown that the single substitution of moxifloxacin for ethambutol increases antimicrobial activity of the regimen, but this increase is not sufficient to achieve acceptable cure rates after truncation of therapy to four months (Burman et al., 2006; Rustomjee et al., 2008; Conde et al., 2009, Merle et al, 2014, Gillespie et al., 2014).

Quantitative capability of the Xpert MTB/RIF assay and its correlation with smear microscopy and culture.

The Xpert MTB/RIF Assay, endorsed by the WHO in 2010 and FDA approved for marketing in the US in 2013 for diagnosing TB, simultaneously detects the presence of *M. tuberculosis* in sputum and determines if genetic markers for rifampin resistance are present. Two studies have compared Xpert MTB/RIF results with sputum smear results in newly suspected pulmonary TB (Blakemore et al, 2011; Friedrich et al 2011). Medium and high qualitative readings from Xpert MTB/RIF correlate well with finding acid fast bacilli on sputum smears. The quantitative capability of the Xpert MTB/RIF assay will be used in this trial at baseline to permit participant randomization based on a medium or high semi-quantification of *M. tuberculosis* copies on their Xpert test at screening. Drug susceptibility results as provided by the Xpert MTB/RIF assay or the Hain MTBDR*plus* assay, will also be used as part of screening and enrollment procedures.

Efavirenz and Rifapentine Drug-Drug Interactions

Rifamycin antibiotics such as rifapentine have the potential to cause significant drug-drug interactions with antiretroviral therapy. Rifapentine is a known inducer of various cytochrome P450 iso-enzymes. The nonnucleoside reverse transcriptase inhibitor efavirenz is a cytochrome P450 substrate, leading to concern for decreased efavirenz concentrations and an increased risk of virologic failure if dosed concurrently with rifapentine. Exposure-response relationships between efavirenz concentrations and virologic failure have been demonstrated. For example, Cohen et al. reported that in an evaluation of 142 HIV-infected persons, efavirenz mid-dosing interval concentrations <1 mg/L were strongly associated with an increased risk for virologic failure (odds ratio 12.5, 95% CI, 2.7-57.3) (Cohen, 2009). The collective data for efavirenz indicate an increased risk of virologic failure if mid-dosing interval (or trough) concentrations are less than 1 mg/L.

Clinical studies evaluating the effects of rifapentine co-administration on efavirenz pharmacokinetics and efficacy have shown mixed results. Among individuals with HIV infection enrolled in a clinical trial of treatment for latent TB infection, preliminary data recently presented by Podany et al. suggest no clinically relevant increase in efavirenz oral clearance when dosed together with isoniazid and rifapentine (10 mg/kg) once daily for four weeks. In this study of 87 patients, median mid-dosing interval efavirenz concentrations decreased in the presence of rifapentine (2588ng/mL vs 2460ng/mL), suggestive of an induction effect on efavirenz by rifapentine; however, the geometric mean ratio of efavirenz oral clearance

increased by only 4%. Additionally, virologic suppression was maintained in 97% of the patients in the study (Podany, 2014). A second study, from Farenc et al. investigated the effect of repeated once weekly 900mg rifapentine dosing on efavirenz pharmacokinetics. In 12 HIV-infected, TB free adults, the authors found a minimal decrease in efavirenz exposure, as measured by a mean decrease in AUC_{0-24} of 14% (Farenc, 2014). A third study investigated the effect of daily rifapentine dosing (15mg/kg) for 21 days in HIV-infected, TB free adults receiving efavirenz based ART with baseline suppressed viral load (VL< 20 copies/mL). After a single dose of rifapentine efavirenz PK was unchanged (Cmax, AUC_{0-24}, Cmin). However, after 21 once-daily doses of 15 mg/kg rifapentine, decreases of 17%, 37% and 33% were seen in Cmax, AUC_{0-24} and Cmin respectively (Sanofi, 2014). All patients maintained viral suppression while taking RPT. While these studies are encouraging in that co-administration of rifapentine at daily doses of 10 and 15 mg/kg with EFV-based ART did not appear to increase risk of HIV treatment failure, further investigations of efavirenz PK and HIV treatment response when rifapentine is given at 1200 mg daily for a longer duration are needed.

The Tuberculosis Trials Consortium (TBTC)
The mission of the TBTC, funded by the U.S. Centers for Disease Control and Prevention, is to conduct programmatically relevant clinical, laboratory and epidemiologic research concerning the diagnosis, clinical management, treatment and prevention of tuberculosis infection and disease. The TBTC has sites in the United States, Spain, South Africa, Hong Kong, Kenya, Vietnam, Peru, and Uganda. All sites have close connections with the local TB control program; some sites are based in the TB control program. All sites work with experienced mycobacterial laboratories, and the CDC's Mycobacteriology Laboratory serves as the central laboratory for confirmation of drug-susceptibility testing, DNA fingerprinting, and further characterization of drug-resistant isolates. Since its inception in 1994 over 13,000 patients have been enrolled in TBTC clinical trials.

The AIDS Clinical Trials Group (ACTG)
The mission of the ACTG, established in 1987, is to develop and conduct scientifically rigorous translational research and therapeutic clinical trials, in the U.S. and internationally, related to HIV infection and its complications including tuberculosis. The ACTG is funded by the U.S. National Institutes of Health through the National Institute of Allergy and Infectious Diseases. ACTG units and investigators serve their communities as major resources for HIV/AIDS research, treatment, care, and education. The ACTG tuberculosis laboratory infrastructure consists of one international tuberculosis specialty laboratory as well as regional tuberculosis diagnostic laboratories.

2.2 Rationale

Rationale for regimen selection
The current standard six-month TB treatment regimen for drug-susceptible pulmonary tuberculosis is associated with unacceptably high rates of treatment default under program conditions, thereby contributing to individual morbidity and mortality, *M. tuberculosis* transmission, and drug resistance. Highly potent regimens of shorter treatment duration may facilitate treatment completion and direct observation of treatment, thereby improving individual and public health. Studies using animal models of TB chemotherapy have shown a clear relationship between rifamycin exposure and reduction of bacillary burden. Animal studies also indicate that rifapentine-based regimens are highly potent and can reduce overall tuberculosis treatment duration to approximately 3 months. In humans, phase 1 and 2 clinical trials support the safety and tolerability of rifapentine at daily doses up to 20 mg/kg. A phase 2 clinical trial has shown a strong drug exposure-response effect for rifapentine using a surrogate marker of time to stable culture conversion. These results provide rationale for a phase 3 clinical trial to determine the

efficacy, using the definitive endpoint of durable cure, of a regimen containing rifapentine substituted for rifampin and administered in combination with other drugs for 17 weeks (approximately four months).

This trial will also assess the efficacy of a second investigational 17-week regimen that incorporates two strategies to enhance antimicrobial activity. The first strategy is optimization of rifamycin exposure through the single substitution of high dose rifapentine for rifampin throughout treatment as described above. The second strategy is a dual substitution approach that seeks to further enhance the potency of the regimen by also replacing ethambutol (which has relatively weak activity) with moxifloxacin, in the context of optimized rifamycin exposure. As described above, available evidence from animal models and in humans indicates that moxifloxacin, when substituted for ethambutol, will contribute to regimen bactericidal activity, even though that single substitution alone is insufficient to shorten treatment to four months. The investigational regimen that contains both rifapentine and moxifloxacin may well be the most potent regimen possible without new chemical entities. Therefore if both investigational regimens fail in the proposed study, then the implications are that new drugs are required for treatment shortening, and that treatment shortening cannot be achieved with existing drugs. Such a finding would push the drug-susceptible TB therapeutics field in a different direction.

Rationale for dosing strategy
With respect to rifapentine dose and dosing strategy, this trial will use a flat dose of 1200 mg with food dosed daily. This is based on 1) demonstration of safety of rifapentine at 1200mg in phase 1 and 2 trials, 2) demonstration that body weight does not significantly affect rifapentine clearance, 3) recognition of an effect of food in substantially increasing rifapentine absorption (Zvada et al., 2010) and 4) modeling predictions that the target rifapentine exposure (AUC of approximately 500 to 600 mcg*h/L) is achievable using this strategy.

For rifampin, administration with food slows the rate of absorption and decreases the maximal concentration (Cmax) by about 36% in healthy adults but to a lesser extent (5 to 15%) in TB patients, with little to no effect on AUC (reduction of 6% in healthy adults; reduction of 4% to increase of 8% in TB patients) (Peloquin et al., 1999; Zent et al., 1995). Overall the clinical consequences of these PK effects are unclear. As a consequence rifampin will not be dosed with food.

Rationale for an open-label (not blinded) study
This study will be open-label; participants, study staff, and clinical care providers will have knowledge of treatment assignment. While blinding is often incorporated into clinical trials as a strategy to reduce bias, blinding is not without cost. Besides logistic complexity, there are two main reasons to not incorporate blinding into this trial. First, if blinding through use of placebos were to be incorporated into this trial, the already substantial pill-burden would be further increased to approximately 20 pills per day, which will be difficult for participants to tolerate. Poor tolerability of the pill burden may result in diminished adherence to treatment and in turn efficacy. Besides tolerability, from a biological perspective the dissolution of pills in the gastrointestinal tract may be reduced with such a high pill burden, a situation that could result in diminished treatment efficacy and increased risk for acquisition of drug resistance. Reduced bioavailability due to the burden of added placebo pills also might reduce the generalizability of study findings to possible subsequent programmatic usage. As noted above, there may be a differential effect of food on rifampin and rifapentine such that different food advice is required so as to optimize regimen pharmacokinetics and potential efficacy. The study will minimize any impact of ascertainment bias through the use of objective laboratory measures for the primary efficacy endpoint as well as incorporation of a central study clinician (who is blinded as to individual participants' treatment assignments) to be available to advise on protocol procedural issues. Co-intervention bias is unlikely during the active treatment in the first four months, and it is unlikely that the use of placebo can effectively

avoid contamination bias in the last two months. Co-intervention bias is unlikely during active TB treatment, as site teams will closely monitor participants. Measures will be taken to evaluate participants equally, regardless of assigned study arm, such that timing of study visits and endpoints will be applied uniformly.

Rationale for CD4 testing before enrollment

HIV-infected individuals will be excluded from enrollment if, at the time of enrollment, their CD4 T cell count is known to be <100 cells/$mm^{3.}$ The rationale for doing so is the challenge of starting concomitant treatment for HIV-infection and TB within a short period of time as recommended by WHO guidelines, the potential for severe immune reconstitution when antiretroviral therapy is initiated early in the course of anti-TB therapy, an increased probability for the diagnosis of conditions that would require therapy with medications that have drug-drug interactions with study drugs, and the indication for primary antimicrobial prophylaxis against opportunistic infections (such as toxoplasmosis and *Mycobacterium avium*) with consequent increase in the probability of drug-drug interactions and adverse events. Additionally, treatment assignment is stratified by CD4 T cell count (Section 7.2).

Rationale for including adolescents

Inclusion of children in clinical trials of tuberculosis treatment increasingly has been called for to support the rational use and increased availability of anti-tuberculosis medications for children (Burman 2006, McKenna 2014). Tuberculosis disease characteristics, presentation, diagnosis, and treatment are similar for adults and adolescents.

Rifapentine currently is approved in the United States for use in persons as young as 12 years old (Priftin® package insert). Rifapentine pharmacokinetic results have been found to be similar between adults and adolescents down to this age (Marshall 1999). Rifapentine 900 mg once weekly, with isoniazid for 12 weeks has been used to treat children for latent TB infection (LTBI) (Villarino 2015): the rifapentine-containing regimen was found to be as safe and effective as a 9-month daily isoniazid regimen, among 552 children age 2-17 years old treated with the rifapentine-containing regimen. Based on a large clinical trial that reported treatment of patients as young as 12 years old (Sterling 2011), CDC recommends use of this rifapentine-containing regimen “as an equal alternative to 9 months of daily self-supervised INH for treating LTBI in otherwise healthy patients aged ≥12 years.” (CDC 2011) Although the phase 2 trial of daily high-dose rifapentine enrolled 81 participants ≧ 18 years old (Dorman 2013), available animal and human data and a formal review by Sanofi support the safety of including adolescents in this clinical trial (Marilyn Maroni personal communication).

Moxifloxacin usage in children has been limited because of findings that treating juvenile dogs with doses higher than recommended for humans (≥ 30 mg/kg/day) resulted in arthropathy (Avelox® package insert). Moxifloxacin has been recommended and used in children for treatment of multidrug resistant (MDR) TB although few safety data have been collected systematically (Bradley 2011). One recent report of a series of 9 children age 6 months to 13 years, treated with moxifloxacin for a range of 3-16 months, attributed side effects possibly to moxifloxacin in 2 patients (Garazzino 2014): A 6-year old girl developed arthritis of the ankle after 3 months, which “spontaneously resolved few days after drug cessation.” A 3-year old girl developed “grade three elevation of liver function tests after 9 months of treatment.” A more recent report of 23 children, median age 11.1 years (IQR 9.2-12.0 years), treated for MDR TB with moxifloxacin followed the children for a median of 236 days (IQR 142-541 days (Thee 2014). This group found lower exposures in children than in adults following an oral dose of 10 mg/kg and lower exposure with HIV infection. They found moxifloxacin to be safe and well tolerated, with a conservative approach to determining an event not to be related to moxifloxacin in the presence of multiple second line drugs.

Adverse events possibly, probably, or definitely related to moxifloxacin were arthralgia (4 patients, 5 events, 4 grade 1, 1 grade 3), pain other than traumatic injury (1, grade 1), headache (5 patients, 5 events, 4 grade 1, 3 grade 3), fatigue/malaise (1, grade 1), nausea (9 patients, 9 events) grade 1), vomiting (3 patients, 3 events, 3 grade 1),cutaneous reaction (1, grade 1), pruritus,(2 patients, 4 events, 4 grade 1), elevated alanine-aminotransferase (ALT) (4 patients, 5 events, 2 grade 1, 2 grade 2 1 grade 3), bilirubin (1 grade 2). ECG data was available for 13 children: mean QTc was 403 ms (SD 30 ms); none had QTc interval >450ms. In a separate report, a case of self-limited polyarthritis was reported for a 12-year old child who inadvertently received a single 2 gram dose of moxifloxacin (50 mg/kg/day) (Torres 2008), illustrating the presence of some risk but also the difficulty of finding moxifloxacin toxicity in children with standard dose treatment.

Daily treatment of adolescents and children with moxifloxacin and with high-dose rifapentine has been limited. The benefits of finding regimens that would substantially shorten treatment for this important subset of TB patients outweigh the theoretical risks. The frequent careful monitoring for signs and symptoms in a clinical trial provides the opportunity to respond quickly to abnormal findings if they occur in an individual, but also to document systematically the safety profile for these promising treatment regimens.

2.3 Potential Harms and Benefits

2.3.1 Potential harms

Risks associated with study participation include the possibilities that an investigational regimen has efficacy that is inferior to the standard regimen and/or an investigational regimen is more toxic than the standard regimen, and confidentiality risks.

The investigational regimens could prove to have inferior efficacy compared to the standard regimen. TBTC Study 29X showed that a regimen containing rifapentine administered daily at 1200 mg (approximately 20 mg/kg) has sufficient antimicrobial activity to justify further evaluation compared to a regimen containing rifampin administered daily at approximately 10 mg/kg, using a surrogate marker for durable cure. Other recent phase 2 studies have demonstrated that substitution of moxifloxacin for ethambutol increases the antimicrobial activity of the regimen. Equipoise around whether these increases in antimicrobial activity can result in durable cure after four months of treatment forms the basis for inclusion of test regimens 2PHZE/2PH and 2PHZM/2PHM. Further, as described above, studies have shown that moxifloxacin substituted for ethambutol increases antimicrobial activity, and PZA has important bacterial sterilizing properties. The following measures will minimize risk to participants: individual participants will be closely monitored clinically and bacteriologically, an interim efficacy analysis will be performed, and a Data and Safety Monitoring Board will review trial progress regularly. In addition, it is anticipated that participants who are not cured will be re-treatable with conventional anti-tuberculosis drugs as acquired drug resistance is very unlikely in the setting of highly monitored directly observed combination therapy (Johnson et al., 2009, Jindani et al., 2013).

The investigational regimens could prove to have greater toxicity than the standard regimen. This is unlikely given that toxicity data collected in the phase 2 clinical trial showed that rifapentine administered at a once daily dose of 20 mg/kg for eight weeks was well tolerated. However, no clinical trial has included patients treated with daily rifapentine for more than 2 months. With respect to the moxifloxacin-containing regimen, the toxicity profile of moxifloxacin is well known. Moxifloxacin is used regularly for prolonged periods during the treatment of patients with drug

resistant tuberculosis. Participants in this clinical trial will be monitored closely for adverse events. The Data and Safety Monitoring Board (DSMB) will review safety data regularly.

Confidentiality risks will be minimized through the measures described in Section 15.3.

2.3.2 Potential benefits

All 3 study treatment regimens are offered with therapeutic warrant, meaning that it is reasonable to expect that these TB treatments will cure TB at an acceptable, if not yet uniformly demonstrated, rate. Study participants will benefit indirectly as it is well established that tuberculosis outcomes for participants in tuberculosis treatment trials are better than those for patients receiving routine care. This study will benefit society by contributing to the understanding of optimal strategies for treating tuberculosis. The inclusion of clinical specimen storage along with the combined use of two culture media types (solid and automated liquid) in this phase 3 trial will contribute urgently needed data that can help determine the optimal use of liquid culture in drug development and will facilitate discovery efforts for new surrogate markers.

3 DESCRIPTIONS OF STUDY DRUGS

Drugs (commercially available as brand or generic) to be administered during this study include the following: rifapentine, rifampin, moxifloxacin, isoniazid, pyrazinamide, ethambutol, and vitamin B6 (pyridoxine). Rifapentine and the other drugs are described in detail in their package inserts. Key points are summarized below.

3.1 Rifapentine

Rifapentine is indicated for the treatment of pulmonary tuberculosis caused by *Mycobacterium tuberculosis*, and must be used in combination with one or more antituberculosis drugs to which the bacterial isolate is susceptible (Priftin package insert, 2010). Rifapentine, as Priftin® was approved by the U.S. FDA in 1998. FDA approval was based on the results of "Clinical Study 008", an open-label, prospective, randomized study of 722 patients with active pulmonary TB (Priftin package insert, 2010). Rifapentine is a semisynthetic rifamycin derivative with a microbiologic profile similar to that of rifampin. Its structure differs from that of rifampin by the presence of a cyclopentyl ring instead of a methyl group at the piperazinyl moiety. It has a longer half-life than rifampin, and, like rifampin, rifapentine inhibits bacterial RNA synthesis by binding to the β-subunit of DNA-dependent RNA polymerase. Rifapentine is well absorbed from the gastrointestinal tract, with 70% bioavailability; when taken with food, its AUC and C_{max} increase by 43% and 44%, respectively (Priftin package insert, 2010). It reaches peak concentrations in the serum 5 to 6 hours after ingestion. Rifapentine and its 25-desacetyl metabolite are highly protein-bound, 97.7% and 93%, respectively, primarily to albumin. Rifapentine is metabolized by an esterase enzyme found in the liver and blood to 25-desacetylrifapentine, a microbiologically active metabolite that contributes about 40% of the drug's overall activity. For *M. tuberculosis*, the MIC of 25-desacetyl rifapentine is 0.25 mcg/mL, while that of rifapentine is 0.05 mcg/ml. The drug and the active metabolite have half-lives of 14-17 and 13 hours, respectively. The drug is excreted in bile and eliminated in feces. Less than 10% of rifapentine is excreted in the urine as unchanged drug. Rifapentine, like other rifamycins, induces CYP3A4, 2C8, and 2C9, which can lead to more rapid metabolism and clearance of many drugs. Rifamycins are also known to induce the activity of phase II enzymes such as glucuronosyltransferase and sulphotransferase and may reduce levels of drugs metabolized by those pathways. Rifapentine is available as 150 mg tablets. Rifapentine, like other rifamycins, causes red-orange discoloration of body fluids and can stain contact lenses. In clinical trials in which rifapentine was combined with isoniazid and other antituberculosis drugs and administered once or twice weekly, rates of adverse reactions were similar with rifampin and rifapentine, with increased liver aminotransferase activity in about 5% of patients. The only adverse effect that has occurred more often with rifapentine than with rifampin has been hyperuricemia when the drug was given twice-weekly; of note, hyperuricemia was attributed to pyrazinamide that was administered concomitantly. Other adverse reactions that occurred in 1-5% of patients included the following: hemoptysis, dizziness, hypertension, headache, gastrointestinal upset, rash, cytopenias, hematuria, pyuria, and proteinuria (Priftin package insert, 2010).

3.2 Rifampin

Rifampin is a semi-synthetic rifamycin derivative that is highly active against mycobacteria, most gram-positive bacteria, and some gram-negative bacteria. It is bactericidal for both intracellular and extracellular microorganisms. By inhibiting prokaryotic DNA-dependent RNA polymerase, it suppresses the early elongation of the nucleotide chain in RNA synthesis. Rifampin 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 8-20 mcg/ml. The half-life of rifampin varies from 2 to 5 hours, and it is shortened by approximately 20-40% after the first week of daily treatment because of the induction of hepatic microsomal enzymes. The half-life is unaffected by renal impairment but is increased by liver disease or biliary obstruction. Rifampin is deacetylated to an enterohepatically-recirculated active metabolite, and 50% to 60% is excreted in the feces. Up to 30% of a dose is excreted in the urine. Approximately 85% of circulating rifampin is bound to plasma proteins, and is widely distributed throughout the body. Rifampin is a potent inducer of a number of hepatic enzymes involved in the metabolism of drugs and some hormones (Venkatesan, 1992). This enzyme induction causes more rapid elimination (and potential loss of efficacy) of many drugs. In the usual daily doses of 10 mg/kg (maximum 600 mg), rifampin is well tolerated. It often causes harmless but disconcerting red-orange discoloration of tears, sweat, saliva, feces, and urine. Less than 4% of TB patients experience significant adverse reactions to rifampin. Gastrointestinal adverse effects are the most common, and they include epigastric distress, anorexia, nausea, vomiting, cramps, and diarrhea. Hepatitis rarely occurs in persons who have normal baseline hepatic function. The incidence of hepatitis may be increased for older persons and those who have chronic liver disease or alcoholism, but remains substantially lower than that for pyrazinamide or isoniazid. Rifampin can cause a flu-like syndrome of fever, chills, headache, dizziness, and bone pain, although this is uncommon using the 600 mg dose given daily. In a very small proportion of patients the flu-like syndrome is associated with interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. (Rifadin [rifampin] package insert. Sanofi, 2013).

3.3 Moxifloxacin

Moxifloxacin is a fluoroquinolone antibacterial that is distinguished by a methoxy group at the C-8 position and an S,S-configured diazabicyclononyl ring moiety at the C-7 position. The mechanism of action against *M. tuberculosis* is by inhibition of the DNA gyrase enzyme involved in DNA replication. Moxifloxacin is well absorbed, with a bioavailability of approximately 90% (Avelox package insert, 2012). Pharmacokinetics are linear in the range of 50-800 mg single dose and up to 600 mg once daily dosing over 10 days. Steady state is reached within 3 days. The mean ($\pm$SD) maximum concentration (C_{max}) and AUC values at steady state with a 400 mg once-daily dosage regimen are 4.5$\pm$0.53 mcg/ml and 48$\pm$2.7 µg*h/ml, respectively. T_{max} is approximately 1-3 hours. The mean ($\pm$SD) plasma half-life is 12.1 $\pm$ 3.1 hours. Trough plasma concentration at steady state (400 mg once daily) is 0.95 $\pm$ 0.10 mcg/L. Co-administration with food may slightly prolong T_{max}, and may reduce the C_{max} by 16%; these effects are not believed to be clinically significant, and thus moxifloxacin can be administered with or without food. Moxifloxacin is 50% bound to plasma proteins. It is widely distributed, with some tissue concentrations reported in excess of plasma levels. Moxifloxacin is metabolized by glucuronide and sulfate conjugation. The cytochrome p450 enzyme system is not involved in the metabolism of moxifloxacin, nor does the drug effect it. Specifically, moxifloxacin does not affect CYP3A4, CYP2D6, CYP2C9, CYP2C19, or CYP1A2. Moxifloxacin should not be administered at the same time as antacids containing magnesium and/or aluminum, sucralfate, antidiarrheals that contain kaolin, or iron and/or zinc containing medications or supplements. As a class, fluoroquinolones are considered to be safe and relatively free of serious adverse effects (Ball, 1989). The safety and efficacy of moxifloxacin for the treatment of community acquired bacterial infections has been extensively studied. In the usual daily doses of 400 mg/day, moxifloxacin is well tolerated. Expected minor adverse events have occurred with a frequency similar to that for other fluoroquinolones in similar populations. Gastrointestinal adverse effects are the most common, and they include nausea (7%), diarrhea (6%), dizziness (3%), abdominal pain (2%), vomiting (2%), dyspepsia (1%), and taste perversion (1%). There are no current reports of hepatitis, although abnormal liver function tests have been noted in 1% of patients. Of note, fluoroquinolones are generally used short-term for acute conditions. However, fluoroquinolones have been found to be safe and

effective in long term use for chronic infections such as osteomyelitis, prostatitis, or chronic urinary tract infection, or skin and skin structure infections (Ball, 1989; Segev et al., 1999). In addition, Valerio et al. found that the combination of moxifloxacin, isoniazid, and rifampin given for 6 months was well tolerated (Valerio et al., 2003). For treatment of MDR-TB, a later-generation fluoroquinolone such as moxifloxacin is recommended for a duration of 8 months or more (WHO, 2011). Moxifloxacin causes a mild prolongation of the corrected QT (QT_c) interval. The mean effect on QT_c interval in 787 patients in Phase 3 clinical trials was 6 $\pm$ 26 milliseconds, but no morbidity or mortality was attributable to QT_c prolongation. In a review of over 6,000 patients treated with moxifloxacin, there were no reports of changes in the frequency of QT_c prolongation (Ball, 2000). In over 10 million patients treated with moxifloxacin there has been no evidence for an increased incidence of ventricular arrhythmia when compared to the overall population. Finally, the incidence of sudden death and syncope, both markers for sudden cardiac events, is not increased when compared to the incidence on the general population. For these reasons, electrocardiographic monitoring is no longer recommended for clinical trials involving moxifloxacin. In this study, moxifloxacin will not be used among patients with a known history of prolongation of the QT interval, patients with uncorrected hypokalemia and patients receiving class Ia (e.g. quinidine, procainamide) or class III (e.g. amiodarone, sotalol) antiarrhythmic agents, due to the lack of clinical experience with the drug in these patient populations (see Eligibility criteria). Pharmacokinetic studies between moxifloxacin and other drugs that prolong the QT interval such as cisapride, erythromycin, antipsychotics and tricyclic antidepressants have not been performed. An additive effect of moxifloxacin and these drugs cannot be excluded, therefore moxifloxacin should be used with caution when given concurrently with these drugs. The estimated incidence of fluoroquinolone-induced tendinopathy is 15 to 20 per 100,000. Fluoroquinolone-induced tendinopathy is diagnosed by a sudden onset of swelling and tenderness concurrent with or shortly after fluoroquinolone therapy, which is accompanied by tendon rupture in about 33% of all cases. The main site affected is the Achilles tendon, though tendinitis has been reported to involve the shoulder, knee, hand, and plantar aponeuroeses. Achilles tendon ruptures have been noted even months after discontinuation (Pierfitte et al., 1996). Concomitant use of corticosteroids is considered to be a risk factor for developing tendinopathy while taking fluoroquinolones. In over 10 million patients treated with moxifloxacin only 3 patients with tendon rupture have been reported. All had received concomitant corticosteroid treatment.

3.4 Isoniazid

Isoniazid is the hydrazide of isonicotinic acid and is one of the primary drugs for TB treatment. The activity of isoniazid is limited to the mycobacteria of the *M. tuberculosis* complex; it is bactericidal for rapidly dividing organisms and bacteriostatic for “resting” bacilli. The probable mechanism of action is the inhibition of the biosynthesis of mycolic acids, a component of the mycobacterial cell wall. Isoniazid is generally well absorbed; food and antacids decrease the rate, but not the extent of absorption. Peak blood levels of isoniazid, 3 to 5 mcg/ml, are obtained 30 minutes to 2 hours after ingestion of routine doses (Peloquin et al. 1999). It diffuses into all body fluids and cells and penetrates into the caseous material of a tuberculoma or pulmonary cavity. In the liver, it is acetylated to inactive metabolites, and 75% to 95% of the dose is excreted as inactive metabolites in the urine within 24 hours. Isoniazid clearance rates depend on 2 metabolic phenotypes, slow and fast acetylation, which are associated with race, but not gender (Ellard., 1984). The isoniazid AUC among persons who have fast acetylation is 30% to 50% of that among persons who have slow acetylation. Because isoniazid is well tolerated over a wide range of therapeutic doses, a single dose per body mass is recommended. Persons who have rapid acetylation achieve effective concentrations, while persons who have slow acetylation do not experience increased toxicity. Half-life ($t_{1/2}$) may vary from 1 hour in fast acetylators ($t_{1/2}$ < 90min) to 3 hours in slow acetylators ($t_{1/2}$ >90min). The usual adult dose of isoniazid is 5 mg/kg given once daily, up to a maximum

of 300 mg given once daily. Isoniazid decreases the clearance of some medications that are metabolized in the liver (particularly carbamazepine, phenytoin, and diazepam) (Baciewicz et al. 1985). However in the context of multidrug therapy including rifampin, these potential drug-drug interaction are of little significance because the effect of isoniazid is counteracted by the more potent opposing effect of rifampin (Kay et al. 1985). The total 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 it is uncommon (<0.2%) at conventional doses. The risk of peripheral neuritis increases for persons who are malnourished or predisposed to neuritis by other illnesses. Concomitant administration of pyridoxine (vitamin B_6) is recommended for these persons, and will be given to all patients in this trial. 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 and occurs in 10-20% of persons receiving isoniazid. However, idiosyncratic severe hepatic reactions are uncommon but are more likely in older persons (up to 2.3% hepatitis incidence in persons more than 50 years old), and may be life threatening. Daily consumption of alcohol increases the risk of isoniazid-associated hepatotoxicity by approximately 4-fold.

3.5 Pyrazinamide

Pyrazinamide is an analog of nicotinamide and has unique activity against *M. tuberculosis*, allowing the duration of treatment to be decreased from 9 months to 6 months (assuming a rifamycin is used throughout). The mechanism of action of pyrazinamide remains incompletely understood, but there is evidence that pyrazinamide inhibits *M. tuberculosis* trans-translation. Pyrazinamide is well absorbed from the gastrointestinal tract and widely distributed into all tissues (Ellard et al., 1987). Usual doses are 15-30 mg/kg/d, up to 2 gm/d. Peak serum concentrations of about 45 mcg/ml are achieved approximately 2-3 hours after a dose. Food and antacids do not significantly affect the absorption of pyrazinamide. The half-life of pyrazinamide is approximately 9-10 hours, and is prolonged in the presence of hepatic insufficiency (Lacroix et al., 1990). Pyrazinamide is metabolized to pyrazinoic acid by the hepatic microsomal enzyme pyrazinamide deamidase. Approximately 40% of a dose is recovered in the urine as pyrazinoic acid and an additional 4% is excreted in the urine as the unchanged parent drug (Ellard, 1969). The remaining drug is thought to be excreted in the bile. There are no known clinically significant drug-drug interactions involving pyrazinamide. The most frequent side effects are skin rash, gastrointestinal intolerance, hepatotoxicity (1.3%), arthralgias (1-7%), hyperuricemia due to blockade of urate excretion (up to 66%), and rarely acute gouty arthritis (Patel et al., 1995; Ormerod et al., 1996). These side effects are seldom dose-limiting. Asymptomatic elevations in serum uric acid are frequent, usually occur during the first or second month of treatment, and are self-limited and require no specific treatment (Zierski et al., 1980). Minor arthralgias also may occur during pyrazinamide treatment and can usually be treated with salicylates or non-steroidal inflammatory agents such as indomethacin while continuing the drug. The most common serious side effect of pyrazinamide is hepatotoxicity. In 2 randomized clinical trials the addition of pyrazinamide to RH did not increase the rates of hepatotoxicity above that seen with the latter 2 drugs alone (Zierski et al., 1980; Combs et al., 1990). However, 3 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 (Yee et al., 2003; Schaberg et al., 1996; Dossing et al., 1996). Hepatitis risk persists with prolonged use (Reves et al., 2014).

3.6 Ethambutol

Ethambutol is an ethylene derivative of butane that interferes with cell wall synthesis in mycobacteria; other bacteria are uniformly resistant to ethambutol. In the treatment of human TB, ethambutol is effective in preventing the emergence of drug resistant strains, although it has no sterilizing activity at clinically-tolerated doses (Kohno et al. 1992). Ethambutol is well absorbed from the gastrointestinal tract, reaching peak serum concentrations of 3-5 mcg/ml in normal volunteers 2-4 hours after a dose. Food slows absorption and decreases the peak serum concentration by 10-20%, but has no effect on the total systemic exposure. Antacids decrease both the peak serum concentration and AUC, and so should not be administered at the same time. Ethambutol is primarily eliminated by the kidneys as unchanged drug; the serum half-life averages 4 hours. Patients with renal insufficiency are prone to accumulation of the drug and the resultant toxicity. There are no known drug–drug interactions involving 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 (Patel et al., 1995). 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, as is the case in this study.

3.7 Vitamin B6 (Pyridoxine)

Pyridoxine (vitamin B6) is an essential vitamin involved in carbohydrate, fat, protein and brain amine metabolism. Isoniazid competitively inhibits the action of pyridoxine in these metabolic functions and also causes increased urinary excretion of pyridoxine (Snider, 1980). Chronic isoniazid administration can result in pyridoxine deficiency, which may manifest as acral paresthesias due to peripheral, predominantly sensory, axonal polyneuropathy. Isoniazid-associated peripheral neuropathy is dose related and occurs in less than 1% of persons receiving isoniazid at recommended doses (Blumberg et. al, 2003). It occurs more frequently in HIV-infected persons, malnourished individuals, persons with chronic renal failure or diabetes mellitus, pregnant and breastfeeding women, and persons with heavy chronic ethanol consumption. Pyridoxine 25 to 50 mg daily by mouth may be administered to prevent isoniazid-associated peripheral neuropathy.

4 OBJECTIVES

4.1 Primary:

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase, to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

4.2 Secondary:

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and assess biospecimens from consenting participants for the purpose of research on discovery and validation of TB biomarkers
- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among patients with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine.

5 STUDY DESIGN

This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.

As described in subsequent sections, participant safety will be maximized and risks will be minimized by frequent study visits for safety assessments, intensive microbiological monitoring for TB treatment failure and relapse, and periodic review of unfavorable outcome rates by a Data and Safety Monitoring Board.

6 STUDY POPULATION

This will be a multisite international study. Male and female participants who are age 12 or older and suspected to have pulmonary tuberculosis will be enrolled into the study.

Target enrollment is 2500 participants.

Pregnant or breast-feeding women will be excluded from the study because of uncertainties about the safety of rifapentine, moxifloxacin, and pyrazinamide in these groups. The sex, ethnicity, and socioeconomic background of study participants are expected to mirror those of the populations served by local tuberculosis clinics and the populations most affected by tuberculosis worldwide.

Co-enrollment in other therapeutic clinical trials is not allowed.

6.1 Subject Inclusion Criteria

Individuals must meet all of the following inclusion criteria in order to participate in this study:

A. Suspected pulmonary tuberculosis plus one or both of the following: a) at least one sputum specimen positive for acid-fast bacilli on smear microscopy OR b) at least one sputum specimen positive for *M. tuberculosis* by Xpert MTB/RIF testing, with semiquantitative result of 'medium' or 'high' and rifamycin resistance not detected.

B. Age twelve (12) years or older

C. A verifiable address or residence location that is readily accessible for visiting, and willingness to inform the study team of any change of address during the treatment and follow-up period.

D. Women of child-bearing potential who are not surgically sterilized must agree to practice an adequate method of contraception (barrier method or non-hormonal intrauterine device) or abstain from heterosexual intercourse during study drug treatment.

E. Documentation of HIV infection status.

F. For HIV-positive individuals, CD4 T cell count greater than or equal to 100 cells/mm^3 based on testing performed at or within 30 days prior to study entry. HIV-positive individuals will be enrolled in a staged approach as described in Section 8.3.10.1, specifically:

- Group 1 ("EFV1"): receipt of efavirenz-based antiretroviral therapy (ART) for a minimum of 30 days at the time of enrollment AND a documented HIV viral load less than 200 copies/mL at or within 30 days prior to study entry, OR
- Group 2 ("EFV2"): for HIV-positive individuals not on ART at enrollment, planned initiation of efavirenz-based ART before or at study week 8

G. Laboratory parameters done at or within 14 days prior to screening:

- Serum or plasma alanine aminotransferase (ALT) less than or equal to 3 times the upper limit of normal
- Serum or plasma total bilirubin less than or equal to 2.5 times the upper limit of normal
- Serum or plasma creatinine level less than or equal to 2 times the upper limit of normal
- Serum or plasma potassium level greater than or equal to 3.5 meq/L
- Hemoglobin level of 7.0 g/dL or greater
- Platelet count of 100,000/mm^3 or greater

H. For all women who are not surgically sterilized or who do not meet the study definition of post-menopausal, a negative pregnancy test at or within seven (7) days prior to screening

I. Karnofsky score greater than or equal to 60

J. Written informed consent

6.2 Criteria for Exclusion from Enrollment

An individual meeting any of the following exclusion criteria at the time of enrollment or initiation of study drugs will be excluded from study participation:

A. Pregnant or breast-feeding

B. Unable to take oral medications

C. Previously enrolled in this study

D. Received any investigational drug in the past 3 months

E. More than five (5) days of treatment directed against active tuberculosis within 6 months preceding initiation of study drugs

F. More than five (5) days of systemic treatment with any one or more of the following drugs within 30 days preceding initiation of study drugs: isoniazid, rifampin, rifabutin, rifapentine, ethambutol, pyrazinamide, kanamycin, amikacin, streptomycin, capreomycin, moxifloxacin, levofloxacin, gatifloxacin, ofloxacin, ciprofloxacin, other fluoroquinolones, ethionamide, prothionamide, cycloserine, terizidone, para-aminosalicylic acid, linezolid, clofazimine, delamanid or bedaquiline

G. Known history of prolonged QT syndrome

H. Suspected or documented tuberculosis involving the central nervous system and/or bones and/or joints, and/or miliary tuberculosis and/or pericardial tuberculosis

I. Current or planned use within six months following enrollment of one or more of the following medications: HIV protease inhibitors, HIV integrase inhibitors, HIV entry and fusion inhibitors, HIV non-nucleoside reverse transcriptase inhibitors other than efavirenz; quinidine, procainamide, amiodarone, sotalol, disopyramide, ziprasidone, or terfenadine.

J. Weight less than 40.0 kg

K. Known allergy or intolerance to any of the study medications

L. Individuals will be excluded from enrollment if, at the time of enrollment, their *M. tuberculosis* isolate is already known to be resistant to any one or more of the following: rifampin, isoniazid, pyrazinamide, ethambutol, or fluoroquinolones.

M. Other medical conditions, that, in the investigator's judgment, make study participation not in the individual's best interest.

N. Current or planned incarceration or other involuntary detention.

6.3 Criteria for Exclusion after Enrollment ('Late Exclusion')

Microbiological confirmation of drug-susceptible tuberculosis is not expected always to be available at the time of enrollment. Enrolled individuals who are subsequently determined to meet either of the following criteria will be classified as 'late exclusions' and study treatment will be discontinued:

A. Screening, baseline, and Week 2 study visit sputum cultures all fail to grow *M. tuberculosis*.

B. *M. tuberculosis* cultured or detected through molecular assays (Cepheid Xpert MTB/RIF or Hain MTBDR*plus* assays) from sputum obtained around the time of study entry is determined subsequently to be resistant to one or more of isoniazid, rifampin, or fluoroquinolones.

7 ENROLLMENT, RANDOMIZATION, AND MASKING PROCEDURES

7.1 Enrollment Procedures

Individuals who are sputum smear microscopy positive for acid-fast bacilli AND/OR have at least one sputum specimen positive for *M. tuberculosis* with a semiquantitative result of 'medium' or 'high' by Xpert MTB/RIF testing (with rifampin resistance not detected) will be invited to participate in this study. Interested individuals will be provided with information about the study including risks and potential benefits of all study procedures. If site staff are satisfied that the potential participant understands the information and the potential participant is willing, the potential participant will be asked to consent to participate in the study. Study-specific procedures will be initiated only after an individual has provided written informed consent.

7.2 Randomization

This will be a randomized trial. Randomization and treatment arm assignment will be computer-generated centrally by the TBTC Data and Coordinating Center. Randomization will be stratified by site, by the presence of cavitation on chest radiograph at baseline (since cavitation is associated with a decreased rate of microbiological response to TB treatment), and by HIV status (HIV-uninfected vs. HIV-infected). Eligible participants (who meet all of the inclusion criteria and none of the exclusion criteria) will be randomly assigned in a 1:1:1 ratio to the study arms. Random assignment sequences will be generated in a way that limits the imbalance between arms within strata while also ensuring that the sequence is not predictable based on previous assignments.

Cavitation is defined as a gas-containing lucent space at least 1 cm in diameter within the lung parenchyma surrounded by an infiltrate or fibrotic wall greater than 1 mm thick seen on chest radiograph. Cavitation seen only on chest tomography (e.g. CT), if done, does not satisfy this definition. Cavities should be distinguished from pulmonary cysts, which are usually thin walled, well-marginated lesions.

8 STUDY PROCEDURES

8.1 Clinical Evaluations

Clinical evaluations will be performed in accordance with a detailed Manual of Operating Procedures (MOOP).

8.1.1 Interview for demographic and contact information

Participants will be interviewed for demographic information including place of birth and date of birth. Contact information will be obtained, including participant location of residence and participant phone number(s), and names and phone numbers of family members/friends who can be contacted by study staff in the event of emergency or if study staff are not able to locate the participant. Identifying and locating information will be maintained only at the site; it will not be entered into the study data base.

8.1.2 Obtaining a medical history

Participants will be interviewed to obtain medical information including tuberculosis signs and symptoms, prior tuberculosis history, other health conditions and treatments (including HIV infection), medicines used, and allergies to medicines.

8.1.3 Obtaining sputum specimens

Sputum may be spontaneously expectorated or induced by aerosol inhalation of sterile nebulized saline. Study sites will use their local procedure for sputum induction. Saliva should not be collected in place of a sputum specimen.

8.1.4 Performing visual acuity and color vision testing

Visual acuity will be tested using a Snellen-type chart. Color vision will also be tested. Results of this testing will not be collected centrally unless a new diagnosis (e.g. ≥ two lines on a Snellen-type chart, or loss of color vision) are discovered.

8.1.5 Symptom assessment

Participants will be asked if they have experienced any of the following within 14 days prior to enrollment or since the previous study visit: fevers, cough, rash, itching, jaundice, nausea, vomiting, diarrhea, loss of appetite, vision problems, numbness/tingling of extremities, joint pain. In addition, participants will be asked if they have had other symptoms not listed above, and if yes, then those other symptoms will be recorded and graded.

8.1.6 Assessment for adverse events

Participants will be asked about symptoms and signs, new medical diagnoses, and hospitalizations since the last study visit.

8.1.7 Chest Radiograph

A posteroanterior chest radiograph will be performed at screening and at the end-of-treatment visit (i.e. week 17 or week 26 visit), as well as for participants undergoing assessment for possible poor treatment response.

8.2 Concomitant Medications/Treatments

The use of all non-study drugs (including over-the-counter medications) from 14 days before starting study drugs through the end of study drug treatment will be monitored and recorded.

Antimicrobials with known antituberculosis activity (see MOOP) should not be used during study drug treatment, and any participant who receives more than five consecutive days of one or more of those medications will be classified as being on a non-study regimen. Antimicrobials without significant anti-tuberculosis activity may be prescribed for intercurrent infections at the discretion of the investigator and will be recorded on study forms.

Participants requiring the use of iron-containing supplements, antacids containing aluminum and/or magnesium, sucralfate, and/or antidiarrheals that contain kaolin should take study drugs at least four hours before or eight hours after ingesting those products in order to avoid impaired absorption of study drugs.

Rifamycins induce hepatic enzyme systems that are active in the metabolism of some other drugs. A prominent drug interaction of rifamycins is that involving hormonal contraceptives. Women of child-bearing potential who are not surgically sterilized must agree to practice an adequate method of contraception (barrier method or non-hormonal intrauterine device) or abstain from heterosexual intercourse during study drug treatment.

Another medically important drug-drug interaction is that involving many antiretroviral drugs and the rifamycins, which are potent inducers of the cytochrome P450 system. Nonetheless, in accordance with World Health Organization guidance, antiretroviral therapy is recommended for HIV-infected study participants; a regimen comprised of efavirenz, tenofovir, plus lamivudine or emtricitabine is recommended for use in study participants based on the absence of known clinically significant drug-drug interactions between these antiretrovirals and the rifamycins. Efavirenz, nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors are allowed while a participant is receiving study treatment, provided that the criteria outlined in Section 8.3.10 are met. Guidance with respect to other antiretroviral agents will be provided in the MOOP and will be updated as new information on drug-drug interactions emerges.

Moxifloxacin has been shown to prolong the QT interval on the electrocardiogram in some patients. Study drugs should not be administered concomitantly with quinidine, procainamide, amiodarone, sotalol, disopyramide, ziprasidone, or terfenadine.

Strategies for management of other common drug-drug interactions will be described in the MOOP.

8.3 Laboratory Evaluations

8.3.1 Sputum mycobacterial tests

See Section 11.1 'Summary of Bacteriological Methods'.

8.3.2 Hematology

Blood will be drawn for a complete blood count that includes a white blood cell differential, hemoglobin, and platelet count. This will require approximately 5 mL of EDTA anticoagulated blood.

8.3.3 Biochemistry

Blood will be drawn for measurement of alanine aminotransferase (ALT; serum or plasma), and total bilirubin (serum or plasma); in addition at screening blood (serum or plasma) will be tested for creatinine, potassium, and albumin. This will require approximately 5 mL of blood.

8.3.4 HIV testing

Counseling prior to and following HIV testing, and reporting of HIV testing results will follow local guidelines and regulations at each site. For study purposes, HIV-1 infection is defined as a positive result using any licensed rapid HIV test or any licensed HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit. Confirmation of the initial study test result is required and must utilize a different method than the one used for the initial study assessment. The confirmatory study test must also be licensed and may include western blot, a second antibody test by a method other than the initial rapid HIV and/or E/CIA, HIV-antigen, or plasma HIV RNA viral load. Two or more HIV-1 RNA viral loads of >1,000 copies/mL are also acceptable as documentation of HIV infection. Negative study HIV testing does not require confirmatory testing. Note: the term 'licensed' refers to an FDA-approved kit or, for study sites located in countries other than the United States, a kit that has been certified or licensed by an oversight body within that country.

8.3.5 CD4 testing

Individuals identified as being HIV-infected will have CD4 T cell count testing performed unless the results are available for a test performed at or within 30 days prior to screening. This will require up to approximately 5 mL of blood.

8.3.6 HIV viral load testing

Individuals identified as being HIV-infected will have HIV viral load measured unless the results are available for a test at or within 30 days prior to screening. This will require approximately 5 ml of blood. Testing can be by any method used routinely at the study site as a standard of care test.

8.3.7 Pregnancy testing

Serum or urine testing methods are acceptable.

8.3.8 Diabetes screening

Hemoglobin A1C is the preferred test. If hemoglobin A1C testing is not available at the study site, then either fasting blood glucose (defined as no caloric intake for at least 8 hours) or random blood glucose can be measured.

8.3.9 Pharmacokinetic sampling: Tuberculosis Drugs

8.3.9.1 Overview

In the context of this overall study, two types of pharmacokinetic (PK) sampling will be used: 'sparse' sampling and 'intensive' sampling. The intensive sampling component will be performed among a convenience sample of patients at a few selected sites with capacity to perform this activity; the intensive PK component will be described in a separate protocol and a separate intensive PK informed consent document will be used. Individuals not undergoing intensive PK sampling will have sparse PK sampling, regardless of assigned study arm. Sparse PK is described below and is a component of the main study protocol. Blood specimens will be shipped to a designated laboratory in the U.S. or Europe, where concentrations of TB drugs and their metabolites will be measured.

8.3.9.2 Timing of sparse PK sampling during study participation

The minimum number of study drug doses prior to the sparse PK blood draw is 14 (including standard DOT and supplementary doses). The preferable timing of sparse PK sampling is at the week 2, 4 or 8 visit (when sputum is also collected). However, if dosing schedule or other factors do not permit sampling at one of these study visits, then sampling may be performed any time after 14 study drug doses and no later than the week 8 study visit.

8.3.9.3 Schedule of sparse PK blood samples

All blood samples will be collected in reference to directly observed doses of intensive phase study drugs -- the reference dose of study drugs is preceded by three directly observed study drug doses given approximately 24 hours, 48 hours, and 72 hours prior. Two or three blood samples, 10 ml each, will be obtained over a 9 hour period as specified in the MOOP.

8.3.9.4 Administration of the Reference Dose of Study TB Drugs

The reference dose of study drugs should be taken by the participant in a manner consistent with the way the participant usually takes the study drugs relative to consumption of (i.e. eating or fasting) and type of food. The timing of food relative to the reference dose of study drugs and a brief description of any food and liquids will be recorded. Participants should abstain from alcohol for 48 hours prior to the reference dose of study drugs and until after the last PK blood specimen is collected.

8.3.10 Pharmacokinetic sampling: Efavirenz

A secondary objective of TBTC Study 31 will investigate efavirenz pharmacokinetics in HIV-infected patients randomized to either of the rifapentine containing regimen arms (Investigational

Regimen 2 and 3). Participants receiving efavirenz-based ART and assigned to a rifapentine-containing arm will fall into one of two groups for efavirenz PK evaluation: 1) on efavirenz for 30 days or more at the time of study enrollment AND documented HIV viral load less than 200 copies/mL at or within 30 days prior to study entry (Group 1, "EFV1"); or 2) HIV-infected participants not on ART at enrollment but started on efavirenz-based ART before or at study week 8 (Group 2, "EFV2").

Participants randomized to the control 2RHZE/4RH arm may initiate efavirenz-based ART at or after study entry; initiation of ART within 8 weeks is strongly encouraged in accordance with international practice guidelines. Participants randomized to the control 2RHZE/4RH arm will not have efavirenz PK evaluations.

Efavirenz plasma concentrations will be evaluated early in the study and in a limited number of participants to determine whether or not standard efavirenz dosing (600mg daily) results in adequate efavirenz exposure in the presence of daily 1200mg rifapentine treatment. An initial group of 31 evaluable participants from EFV1 will be enrolled. After the first 31 evaluable participants in EFV1 are enrolled, no further Group 1 participants will be permitted to enroll until data from these first 31 individuals are evaluated and proved to be clinically acceptable. EFV2 participants will not be allowed to enroll into the study until data from 31 evaluable individuals in EFV1 have been evaluated and deemed clinically acceptable, that is, there is no compelling evidence of lack of adequate exposure to efavirenz in EFV1 patients.

The efavirenz PK data by Group will be judged acceptable if we have evidence that >80% of participants have estimated efavirenz mid-dosing interval concentrations **≥**1 mg/L. Any patient that has a baseline (pre-TB treatment) efavirenz concentration <1mg/L will be deemed non-evaluable for the efavirenz pharmacokinetic study. Methods for estimating efavirenz pharmacokinetic parameters are provided in Section 13.5.5. Blood specimens will be shipped to a designated laboratory in the U.S. where concentrations of efavirenz will be measured.

EFV1: The first evaluation will be made when baseline, weeks 4 and 8 efavirenz concentrations are available for 31 evaluable participants; if ≤20 of the 31 have acceptable efavirenz concentrations at both Weeks 4 and 8, the team would consider this to be of concern. This rule was developed to have a high likelihood (95% or higher) of continuing to accrue participants if the true underlying rate of having acceptable efavirenz concentrations is greater than 80%. If accrual or shipping patterns warrant, then an interim analysis prior to assessment of 31 participants may be performed; in this case, an independent statistician who has not seen any PK data will develop the decision rule to protect the original PK study design and participant safety. The second evaluation will occur when 90 participants in EFV1 are enrolled. Additional details will be specified in the statistical analysis plan, including the role of viral load in initial evaluation.

EFV2: Once the efavirenz PK data have been evaluated in the first 31 patients from EFV1 and deemed clinically acceptable (that is, >20 of the 31 in EFV1 have acceptable efavirenz concentrations at both weeks 4 and 8) enrollment will open to patients in EFV2 (HIV-Infected participants starting on efavirenz early after initiation of study tuberculosis drugs). PK assessments in EFV2 will occur in a similar manner as in EFV1 participants with two phases of assessments (N=31 and 90 patients) except that sampling will occur approximately 4 and 8 weeks following initiation of EFV. In addition, HIV-1 viral load measurements will be conducted 8 weeks after starting efavirenz and at study week 22. Stopping rules for numbers of patients with acceptable efavirenz concentrations in EFV2 will be the same as those for EFV1. Additional

details will be specified in the statistical analysis plan, including the role of viral load in initial evaluation.

If stopping rules are met for unacceptable proportions of participants with acceptable efavirenz concentrations at any time (after 31 or after 90 patients in either Group), the TBTC 31 team may consider appropriate action based on study results, including whether to advise adjusting efavirenz dose or to exclude efavirenz use in subsequent study participants. If dose adjustment of efavirenz is recommended the step-wise PK evaluations will continue in a similar fashion as with the 600mg dose to again determine if acceptable efavirenz concentrations are maintained with the increased dose. Stopping rules for unacceptable proportions of patients with less than 1mg/L concentrations of efavirenz will be the same as with the 600mg dose.

8.3.10.1 Efavirenz Sampling Strategy

8.3.10.1.1. HIV-infected participants on efavirenz at the time of study enrollment (EFV1): Blood (approximately 6 ml) for measuring plasma efavirenz concentrations will be obtained at screening or at baseline (prior to initiation of study tuberculosis drugs) and additionally at weeks 4, 8 and 17 while on efavirenz. Blood will be drawn approximately 12 hours (no sooner than 10 hours after and no later than 24 hours) after the previous dose of efavirenz was ingested. The time of that efavirenz dose and the time of blood collection will be recorded. HIV viral load testing will also be performed at weeks 8 and 17; if a follow-up viral load result is greater than 200 copies/mL then, approximately 2 to 4 weeks after that test, blood should be drawn and a repeat HIV viral load test performed.

8.3.10.1.2. HIV-infected participants started on efavirenz after initiation of study tuberculosis drugs (EFV2):

Blood (approximately 6 ml) for measuring plasma efavirenz concentrations will be obtained at about 4 and 8 weeks following initiation of efavirenz (to coincide with study visits to the extent possible – see recommended schedule below), and in addition at the study week 22 visit. Blood should be drawn approximately 12 hours (no sooner than 10 hours after and no later than 24 hours) after the previous dose of efavirenz was ingested. The time of that efavirenz dose and the time of blood collection should be recorded. HIV viral load testing will be performed at 8 weeks following initiation of efavirenz (to coincide with study visits to the extent possible – see recommended schedule below) and at study week 22; if the week 22 viral load result is greater than 200 copies/mL then, approximately 2 to 4 weeks after that test, blood should be drawn and a repeat HIV viral load test performed.

EFV2.A. If efavirenz is initiated at or within one week after initiation of study TB drugs: obtain efavirenz sample at study week 4, study week 8, and study week 22; also obtain HIV viral load at study week 8 and study week 22

EFV2.B. If efavirenz initiated between weeks 1 and 4 of study TB treatment: obtain efavirenz sample at study week 8, study week 12, and study week 22; also obtain HIV viral load at study week 12 and study week 22

EFV2.C. If efavirenz initiated between weeks 5 and 8 of study treatment: obtain efavirenz sample at study week 12, study week 17, and study week 22; also obtain HIV viral load at study week 17 and study week 22

8.3.11 Pharmacogenomic testing: Host Genetic Analysis

This study will evaluate human gene polymorphisms that may affect pharmacokinetics of tuberculosis drugs and antiretroviral drugs. These include polymorphisms in SLCO1B1 (which may affect pharmacokinetics of rifamycins), NAT2 (which affects pharmacokinetics of isoniazid), and cytochrome P450 isoenzyme 2B6 (CYP2B6) (which affects pharmacokinetics of efavirenz). Data from these genetic assays may be used as covariates or for stratification in analyses of study endpoints. Consent will be obtained specifically for genetic testing; participants may opt out of genetic testing and still participate in the treatment study. For consenting participants, blood (approximately 8.5 ml) will be collected once after enrollment as specified in the MOOP. Blood specimens will be shipped to a designated laboratory in the U.S. for analysis.

8.3.12 Storage *of M. tuberculosis* bacterial isolates

The *M. tuberculosis* bacterial isolates from screening and baseline, as well as isolates cultured from specimens obtained at or after week 17 will be stored. Isolates should be labeled with study identification number and collection date, and not with participant name. Isolates will be shipped to the CDC TB reference laboratory upon request. These isolates will be used for confirmatory drug susceptibility testing, for study endpoint assessment and for genetic characterization of *M. tuberculosis.*

8.3.13 Samples for analyses to identify potential biomarkers of tuberculosis treatment response

At participating sites, sputum, urine, and blood will be collected from consenting participants for the purpose of research to identify potential biomarkers of tuberculosis treatment response. Specimens collected for storage will be labeled with study identification number and collection date, and will not be labeled with participant name. Specimens will be centrally stored at Biomedical Research Institute, Rockville, MD, USA. Consenting patients will be asked to provide sputum, blood (approximately 8 ml blood per blood draw), and urine samples at 5 visits (baseline, week 2, week 4, week 8, and at end of treatment). Additionally, samples should also be collected if a) failure or relapse is suspected and evaluated at an unscheduled visit or a visit other than one of the 5 visits indicated above; and/or b) the participant voluntarily withdraws from the study. Participant consent for collection and storage of specimens will be part of the study Informed Consent Form which will be administered during the Screening/Consenting visit, with an independent signature line. As an alternative to the sample storage plan described above, participants enrolled in this clinical trial will be permitted to co-enroll in The Platform Study Biobank Substudy (TBTC Study 36A) at TBTC sites or the Biobank for Surrogate Marker Research for TB (A5302) at ACTG sites, which includes additional sample types and additional timepoints for collection, requiring a separate biobanking informed consent document.

8.4 Specimen preparation, handling and shipping

Specimen preparation and handling will be performed according to procedures set forth in the MOOP. Specimens will be labeled with study identification number and not with participant name. Shipping will be performed by trained personnel according to the Manual of Procedures and in accordance with local and international regulations.

8.5 Loss to follow-up

All efforts should be made to contact participants that miss DOT visits or scheduled study visits (unless the participant has withdrawn consent).

For participants who fail to attend a scheduled study visit during study treatment and attempts to reach the participant by phone are not successful or not feasible, a home visit will be performed within one week of the scheduled study visit.

For participants who fail to attend a study visit during study follow-up (off study treatment) and attempts to reach the participant by phone are not successful or not feasible, a home visit will be performed within approximately two weeks of the scheduled study visit. If a participant misses their final scheduled visit then repeated efforts must be made to contact the participant, until six weeks after the Month 18 visit of the Last Participant In. If these attempts are unsuccessful then the participant should be considered lost to follow-up and reported as such.

8.6 Participants with a positive sputum culture for *M. tuberculosis* at or after week 17

In the event of a positive culture confirmed as *M. tuberculosis* from a sputum specimen obtained at or after week 17, procedures described in Section 9.8 “Study Procedures for Participants with Possible Poor Treatment Response” should be followed.

TB treatment change (including restarting treatment) is usually not indicated by a single positive culture, and is usually not considered unless there is worsening of clinical signs/symptoms and/or chest X-ray findings. If the participant remains well clinically (i.e. there is no clinical or radiological evidence of failure or relapse), then follow-up off treatment should be considered, while culture results are pending.

8.7 Participants with a positive sputum smear at or after week 17

In the event of a positive sputum smear from a specimen obtained at or after week 17, procedures described in Section 9.8 “Study Procedures for Participants with Possible Poor Treatment Response” should be followed.

Sputum smear results alone should not be used for clinical decision-making during the study.

8.8 Participants with a possible poor treatment response

In addition to Sections 8.6 and 8.7, for study purposes a possible poor treatment response evaluation also is triggered by any one or more of the following, but not limited to:

a) Worsening signs and/or symptoms consistent with TB at or after week 17

b) Radiographic worsening consistent with TB at or after week 17

c) The site investigator is considering extension of TB treatment beyond that of the participant's assigned regimen

d) The site investigator is considering retreatment with any TB therapy after the participant has completed assigned study treatment

e) For a participant on assigned study treatment, the site investigator is considering a change in treatment for efficacy reasons (this does not apply to changes in treatment for pregnancy, temporary drug challenge, or toxicity).

In general, for any participants suspected of possible poor treatment response, procedures described in Section 9.8 "Study Procedures for Participants with Possible Poor Treatment Response" should be followed.

8.9 Management of a participant who is discontinued from study treatment

For any participant who is discontinued from study treatment, the assessments listed in Section 9.9 "Post Early Termination Visit" should be performed.

8.9.1 Management of a participant who is discontinued from study treatment because s/he is determined to be ineligible after enrollment ('late exclusions')

These individuals should have study drugs discontinued, and undergo an early termination visit approximately 14 days after discontinuation of study drugs. Individuals should be referred to local sources of medical care.

8.9.2 Management of participants who become pregnant during the study

Women who become pregnant while receiving study therapy will be taken off of study treatment and treated according to National Tuberculosis Program or local guidelines. The women will continue to receive scheduled study follow-up, will be classified as being on a non-study regimen, and will not receive study radiographs. The outcome of the pregnancy will be reported on study forms.

Women who become pregnant while in study follow-up (not on study treatment) will continue to receive scheduled study follow-up, and will not receive study radiographs. The outcome of the pregnancy will be reported on study forms.

8.9.3 Management of participants who are discontinued from study treatment in the setting of an adverse event or the investigator judges that discontinuation of study treatment is in the participant's best interest

The site investigator may discontinue a participant from treatment in the event of a severe or serious adverse event, or at any time if the investigator thinks discontinuation is in the participant's best interest. For study purposes, such participants should continue to be followed in

the study for outcome determination in accordance with the study MOOP, unless the participant withdraws consent. The participant should be referred to appropriate local sources of care for management of medical problems that cannot reasonably be managed by the study team.

8.9.4 Management of a participant who requests premature discontinuation from study treatment

A participant may request premature discontinuation from study treatment. A post early termination visit should be performed and the participant should continue to be followed in the study per the study schedule, unless the participant withdraws consent. The participant should be referred to appropriate local sources of care for management of tuberculosis.

8.9.5 Management of a participant who is incarcerated after enrollment

This study will not enroll prisoners. However, it is possible that a participant will be incarcerated after enrollment. If an enrolled individual is incarcerated, then study medications will be stopped and the participant will be treated for active TB according to the standards of the institution in which s/he is incarcerated. While incarcerated, individuals will not be followed in the study. When the individual is no longer incarcerated, study treatment and/or study follow-up may continue, at the discretion of the investigator.

8.10 Premature termination of the study or closure of a study stratum or a study site

The study or a study stratum can be terminated by the sponsor on the advice of the Data and Safety Monitoring Board. The sponsor has the right to close the study and the sponsor has a right to close a site, although this should occur only after consultation between involved parties. In the event of study termination or site termination, the central and local ethics committees/institutional review boards must be informed. If the study or a study site is closed before the planned end of the study, all study materials (except documents required to be retained and stored on site) must be returned to the sponsor. The site investigator will retain all other documents until notification is given by the sponsor and/or as required by the local regulatory authorities. If the study or a study site is closed prematurely, participants should undergo a Post Early Termination Visit unless otherwise directed by the sponsor.

9 STUDY SCHEDULE

Activities to be conducted at study visits are also shown in Appendix A.

9.1 Screening

The following will be performed after written informed consent:

- Assessment of inclusion and exclusion criteria
- Interview for demographic and contact information
- Medical history
- Height and weight will be measured
- Chest radiograph unless results of a chest radiograph done within the previous fourteen (14) days are available.
- An HIV test will be obtained when any one or more of the following apply: a) HIV serostatus is unknown; b) the last documented negative HIV test was > 3 months prior to screening; c) written documentation of HIV-1 infection at any time prior to study entry is not available for confirmation of HIV-infected status.
- For individuals known or suspected to be HIV-infected, CD4 T cell enumeration should be performed unless the results are available for a test performed within 30 days prior to screening.
- For individuals known to be HIV-infected, an HIV viral load should be performed unless results are available for a test performed within 30 days prior to screening.
- Urine or serum pregnancy test for all women who are not surgically sterilized or who do not meet the study definition of post-menopausal, unless a negative result is available for a pregnancy test performed within seven (7) days prior to screening.
- A sputum sample will be obtained and sent to the study laboratory for mycobacterial smear and culture.
- Sputum for rapid molecular test, if available at site
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)
- Diabetes screening.
- Blood will be obtained for a complete blood count (including white blood cell differential, hemoglobin, and platelet count), and serum or plasma will be tested for alanine aminotransferase (ALT), total bilirubin, creatinine, albumin, and potassium, unless results from those tests performed within fourteen (14) days prior to screening are available.

9.2 Baseline

The baseline visit is defined as the visit at which study drug treatment is initiated. A baseline visit will be performed for individuals who meet study eligibility criteria and are willing to participate in the study. The baseline visit should be performed as soon as possible and up to 7 days after screening. If applicable the screening and baseline activities may be performed in the same visit, with at least two sputa sent to the

study laboratory. The baseline visit is defined as the visit during which study drug treatment is initiated. Participants who no longer meet eligibility criteria will be referred to a local source of tuberculosis care, and to other appropriate sources of clinical care as applicable. A log will be maintained of individuals who are screened but not enrolled.

The following will be performed at baseline:

- Review of interval tuberculosis treatment to assess whether the participant continues to meet eligibility criteria
- Review of concomitant medications to assess whether the participant continues to meet eligibility criteria
- Review of participant personal contact information
- Symptom assessment
- Concomitant medication assessment
- Measurement of weight
- Visual acuity and color perception testing
- At least ONE SPUTUM sample will be obtained and sent to the study laboratory for smear and culture.
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)
- For participants consenting to collection of specimens for identification of potential biomarkers of TB treatment response: sputum, urine and blood specimens will be obtained..
- For HIV-infected participants on efavirenz and rifapentine treatment (EFV1): plasma for efavirenz quantification (prior to taking study TB drugs)
- Randomization

9.3 Study Visits at Weeks 2, 4, 8, and 12

Study visits should be performed within a window of +/- three (3) days. The following will be performed at each study visit:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- At least ONE SPUTUM obtained for mycobacterial smear and culture
- Measurement of weight
- In addition, at week 4 for all participants: testing of visual acuity and color perception should be performed.

- In addition for EFV1 group HIV-infected participants on efavirenz and a rifapentine regimen: plasma for efavirenz PK at weeks 4 and 8, as well as HIV viral load testing at week 8.
- For EFV2 group HIV-infected participants, see Schedule in Section 8.3.10.1.2
- At weeks 2, 4, and 8 (not at week 12), for participants consenting to collection of specimens for identification of potential biomarkers of TB treatment response: sputum, urine and blood specimens will be obtained.
- In addition, any time after completion of 14 study drug doses and no later than the week 8 study visit for all participants: PK sampling for TB drugs
- In addition, once any time after enrollment for all participants: blood for pharmacogenomic testing

9.4 Week 17

This visit should be performed within a window of +/- three (3 days). The following will be performed:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- At least TWO SPUTA will be obtained for mycobacterial smear and culture
- Chest radiograph (End-of-Treatment) for participants assigned to rifapentine regimens (Regimens 2 or 3)
- Measurement of weight
- In addition for EFV1 group HIV-infected participants on efavirenz and a rifapentine regimen: plasma for efavirenz PK; HIV viral load testing.
- For EFV2 group HIV-infected participants, see Schedule in Section 8.3.10.1.2
- For participants consenting to collection of specimens for identification of potential biomarkers of TB treatment response who in addition have been assigned to one of the 17-week treatment arms: sputum, urine and blood specimens will be obtained.
- For all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.5 Week 22

This visit should be performed within a window of +/- three (3) days. The following will be performed:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- At least TWO SPUTA obtained for mycobacterial smear and culture
- Measurement of weight
- Blood creatinine, ALT, total bilirubin, and complete blood count including WBC differential and platelets
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)
- For EFV2 group HIV-infected participants on efavirenz: plasma for efavirenz PK; HIV viral load testing

9.6 Week 26

This visit should be performed within a window of +/- seven (7) days. The following will be performed:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- At least TWO SPUTA obtained for mycobacterial smear and culture
- Chest radiograph (End-of-Treatment) for participants assigned to rifampin regimen (Regimen 1)
- Measurement of weight
- For participants consenting to collection of specimens for identification of potential biomarkers of TB treatment response who in addition have been assigned to the 26-week treatment arm: sputum, urine and blood specimens will be obtained.
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.7 Study Visits at Months 9, 12, 15, and 18

Study visits should be performed at months 9, 12, 15, and 18. Visits should be performed within a window of +/- seven (7) days. The following will be performed at each study visit:

- Review of participant personal contact information
- Symptom assessment
- Review of interval medical history
- Concomitant medication assessment
- Measurement of weight
- At least TWO SPUTA obtained for mycobacterial smear and culture. For participants whose Month 18 sputa are both contaminated, at least two additional sputa should be obtained.
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.8 Study Procedures for Participants with Possible Poor Treatment Response

The following should be performed as soon as feasible when a possible poor treatment response is suspected, and ideally prior to a change or re-start of treatment (if change or re-start is considered to be warranted by the site investigator) if the participant's clinical condition permits:

- Contact the central study clinician, ideally prior to changing or re-starting TB treatment
- Review of participant personal contact information
- Symptom assessment
- Review of interval medical history
- Concomitant medication assessment
- Measurement of weight
- At least THREE (3) SPUTA should be collected within one week of each other. At least TWO of these sputa should be collected prior to changing or re-starting TB treatment, and at least 4 hours apart. At least ONE sputum should be a first morning specimen, if feasible. These sputa should be sent to the study laboratory for smear and culture. If *M. tuberculosis* is isolated in culture, drug susceptibility testing should be performed on one of the isolates. Then the isolate from each positive culture should be stored frozen.
- Chest radiographComplete the Possible Poor Treatment Response case report form

- For participants consenting to collection of specimens for identification of potential biomarkers of TB treatment response: sputum, urine and blood specimens should be obtained.

Treatment of participants in whom TB treatment is changed or re-initiated should be according to local guidelines. For study purposes, such participants should continue to be followed in the study for outcome determination in accordance with the study MOOP, unless the participant withdraws consent.

9.9 Post Early Termination Visit (Visit after Early Termination) for Participants Terminating before Completion of their Assigned Study Treatment

The early termination visit should occur approximately 14 days after stopping study drugs, in order to assess for late manifesting adverse events. The following should be performed:

- Review of contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- Measurement of weight

The intent is that the procedures for participants with possible poor treatment response occur before stopping study drugs, and that this post early termination visit occurs approximately 14 days after stopping study drugs. Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event (see Section 12). Participants who were receiving study treatment at the time of early termination will be referred to local sources for tuberculosis care. For study purposes, such participants should continue to be followed in the study for outcome determination in accordance with the study MOOP, unless the participant withdraws consent.

A participant who withdraws consent will not have to undergo follow-up study procedures.

9.10 Early Termination Visit for Participants Stopping Study Participation after Completion of their Assigned Study Treatment

Study Procedures for Participants with Possible Poor Treatment Response (Section 9.8) should be followed. In addition, the participant should be asked if they would be willing to have Month 12 and Month 18 study procedures performed.

Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event (see Section 12).

A participant who withdraws consent will not have to undergo follow-up study procedures.

9.11 Missed Visit

A visit is reported as missed if it does not occur within the time frame specified for that visit on the event schedule in Appendix A. Protocol-indicated sputum collection still should be performed within the scheduled visit window, if possible, even if the visit is reported as missed.

9.12 Unscheduled Visit

An unscheduled visit refers to a protocol-required study evaluation that did not occur within the allotted window around the visit date specified on the participant's visit schedule. During an unscheduled visit, all procedures described in the protocol for the particular missed visit should be completed. Study visits that occur solely because of an adverse event or a possible poor treatment response are not considered unscheduled visits.

10 STUDY INTERVENTION/INVESTIGATIONAL DRUGS

10.1 Study Drugs

The study drugs are rifampin, rifapentine, pyrazinamide, ethambutol, isoniazid, moxifloxacin, and pyridoxine (vitamin B6). Each of the study products is described in section 3.

The study products will be combined into the following study treatment regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

Rifampin, isoniazid, pyrazinamide, and ethambutol are the current first-line anti-tuberculosis drugs used worldwide for treatment of drug-susceptible tuberculosis. Pyridoxine (vitamin B6) decreases the risk of isoniazid-related peripheral neurotoxicity and is commonly co-administered with isoniazid during tuberculosis therapy. Each of these agents will be used at conventional doses and dosage schedules during this clinical trial.

Rifapentine, a rifamycin antibiotic, is approved in the United States by the Food and Drug Administration for the treatment of pulmonary tuberculosis; the approved dose is 600 mg twice weekly for two months followed by 600 mg once weekly for four months, in combination with other anti-tuberculosis drugs. As detailed above, pre-clinical and clinical studies indicate that maximal anti-tuberculosis activity is achieved at rifapentine doses higher than 600 mg and intervals shorter than twice weekly. During this clinical trial rifapentine will be administered at a dose of 1200 mg, once daily for 17 weeks in Regimens 2 and 3.

Moxifloxacin is a broad-spectrum fluoroquinolone antibiotic active against *M. tuberculosis*. It is used worldwide for treatment of drug-resistant tuberculosis. During this clinical trial moxifloxacin will be administered at the conventional adult dose of 400 mg once daily, and it will be administered for seventeen weeks in Regimen 3.

10.2 Study Drug Acquisition

All study drugs, except for vitamin B6 for the full duration of study therapy will be provided by Sanofi. Vitamin B6 supplies will be obtained locally. Study drugs will be distributed in bulk to study site pharmacies, which will dispense indicated supplies for individual participants according to individual pharmacy plans that will be approved by the sponsor or the sponsor's designee. Treatment of participants who discontinue study treatment will be with locally supplied drugs.

10.3 Study Drug Storage and Stability

Study drugs will be stored in accordance with the written MOOP, and may not be used after their expiration date.

10.4 Administration and Dosage of Study Drugs

All drugs will be administered orally, seven days per week, throughout treatment. Five of seven doses per week will be given as DOT by study personnel, or by a healthcare worker or lay treatment supervisor who is aware of the study protocol and trained regarding the study protocol. Doses on weekends and on holidays up to three consecutive days may be either DOT or self-administered. Participants receiving a rifapentine-containing investigational regimen should take study drugs within one hour after ingesting food. Participants receiving a rifampin-containing investigational regimen should take study drugs on an empty stomach; for participants on rifampin who have difficulty tolerating study drugs on an empty stomach, then administration with food is reasonable. Information about ingestion of food will be recorded for every study drug dose.

Doses of study medications will be determined by body weight, as follows:

Drug	Dose
Isoniazid	300 mg
Vitamin B6	25 or 50 mg
Pyrazinamide	
< 55 kg	1000 mg
≥ 55-75 kg	1500 mg
> 75 kg	2000 mg
Ethambutol	
< 55 kg	800 mg
≥ 55-75 kg	1200 mg
> 75 kg	1600 mg
Rifampin	600 mg
Rifapentine	1200 mg
Moxifloxacin	400 mg

Drugs and doses used to initiate treatment will be assigned by the enrollment application, based on weight reported at enrollment.

10.5 Dose Modifications for a Participant

10.5.1 Dose Modifications for a Change in Participant's Weight

Study drug doses for pyrazinamide and ethambutol should be adjusted for the participant's weight that was recorded at the most recent scheduled study visit.

10.5.2 Drug Re-challenges

In a participant experiencing side effects, whether to initiate a drug re-challenge as well as the drugs, dosages, and schedule for a drug re-challenge are at the discretion of the treating clinician. General recommendations for consideration are provided within the MOOP.

10.6 Criteria for Discontinuation of Study Drugs

Participants who meet any one or more of the following criteria will be discontinued from study treatment:

- Pregnancy
- Any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued administration of study treatment is not in the best interest of the participant
- Participant request for premature discontinuation of study treatment
- Participants classified as 'late exclusions'

Procedures for study follow-up are described in Section 9. The decision to discontinue a participant from treatment should be discussed with the central study clinician or his/her designee.

10.7 Accountability Procedures for the Study Drugs

Study drugs will be shipped periodically to the study sites. The investigator or designee will acknowledge receipt of and keep an inventory of study drugs. Unused product should be stored at the site until directed by the study sponsor.

10.8 Assessment of Subject Compliance with Study Treatment

Each dose of study drugs given by DOT will be recorded by the healthcare worker. Participants will be asked to record doses of study drugs not administered by DOT. Adherence will be reviewed at each study visit. Non-adherence should prompt an investigation into the cause of non-adherence and measures to address the non-adherence.

11 ASSESSMENT OF EFFICACY

11.1 Summary of Bacteriological Methods

Detailed laboratory procedures for laboratory staff performing mycobacteriological tests are in the MOOP. The following brief description is intended to provide guidance to clinical study staff.

All study sputa (expectorated or induced) should be sent to the designated study site laboratory. Any anticipated changes in the designated site laboratory (i.e. to a different laboratory) and/or a change in the type of solid or liquid media used must be communicated in writing to the TBTC Project Officer prior to executing the change(s).

The following will be performed for all study sputa: Nalc-NaOH decontamination followed by culture using both solid medium and the Becton Dickinson Mycobacterial Growth Indicator Tube (MGIT) liquid culture automated system. Bacterial load will be determined and recorded for all specimens using the time to positivity output automatically provided by the MGIT system.

For all cultures showing growth of acid-fast bacilli, the acid fast bacilli will be species identified at least to the level of *M. tuberculosis* complex vs. not *M. tuberculosis* complex.

For each participant, the first study isolate of *M. tuberculosis* will have culture-based phenotypic drug susceptibility testing performed at the study site-designated laboratory for isoniazid, rifampin, and fluoroquinolones. In addition, phenotypic drug susceptibility testing should be performed for the first of any *M. tuberculosis* isolates obtained at/after the Week 17 study visit.

For each participant, the first study isolate of *M. tuberculosis* as well as any *M. tuberculosis* isolates cultured from sputum obtained at/after the Week 17 study visit should be stored frozen. The first study isolate of *M. tuberculosis* should be sent to the CDC laboratory. Additional *M. tuberculosis* isolates pertinent to the participant's treatment assignment should be sent to the CDC laboratory if requested by the CDC Data Center.

12 ASSESSMENT OF SAFETY

12.1 Specification of Safety Parameters

12.1.1 Primary Outcome Measure

Proportion of participants with grade 3 or higher adverse events during study drug treatment

12.1.2 Secondary Outcome Measure

Discontinuation of assigned treatment for a reason other than microbiological ineligibility

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

12.2.1 Adverse Events and Serious Adverse Events

At each study visit up to and including Week 26, participants will be asked about adverse events that have occurred since their previous visit. Solicited events will include symptoms, hospitalizations, and new medical diagnoses. Participants will also be asked to describe other signs, symptoms, and events not captured by the solicited event listing.

Monitoring of laboratory parameters will be performed as described in Section 9 and Appendix A.

12.2.1.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Any adverse events detected will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) toxicity tables and coded using MedDRA.

12.2.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse event that results in any of the following outcomes:

- Death
- Life-threatening (subject at immediate risk of death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect
- Results in a persistent or significant disability or incapacity
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

12.3 Recording and Reporting Procedures

12.3.1 Overview

A sign or symptom should be reported as an adverse event if it meets either (or both) of the following criteria: a) reaches severity of grade 3 or higher; b) represents a new diagnosis. Laboratory findings should be recorded as adverse events only if clinically significant.

12.3.2 Reporting Procedures

Any SAE or grade 4 toxicity must be reported by the site to the TBTC on an Adverse Event Report within 48 hours of the site's awareness of the event.

SAEs, death, or life-threatening problems *that may reasonably be regarded as caused by or associated with the administration of the study drug regimen* (defined as at least 'possibly associated' with the study drug regimen) must be reported by the site to the Sponsor (TBTC) and the local IRB within 48 hours of the site's awareness of the event. For participants who die, as much information as possible on cause of death and details of the final illness will be obtained from relevant sources.

Any adverse event that meets the definition of being a suspected unexpected serious adverse reaction (SUSAR) will be reported promptly by the Sponsor (TBTC) to the FDA, international regulatory agencies, the CDC IRB, and all clinical investigators. A SUSAR is defined as an adverse reaction that is unexpected (i.e. not consistent with the applicable product information) AND also meets the definition of a serious adverse event AND also may reasonably be regarded as caused by or associated with the administration of the study drug regimen (i.e. at least 'possibly associated' with the study drug regimen).

The DAIDS Medical Officer will receive a copy of the IND safety reports that TBTC submits to the FDA.

12.3.3 Pregnancy

For study purposes, pregnancy must be recorded on an Adverse Event Report form. Management of women who become pregnant during the study is described in Section 8.

12.4 Management of adverse events

For AEs that in the investigator's judgment may be due to study drugs, the following general approach to management should be applied. In general, for grade 1 toxicities, the patient will be followed carefully and the study drugs will be continued. For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs may be temporarily held if in the investigator's judgment continuation of study drugs would be unsafe.

Grade 3 and 4 AEs should be carefully assessed. The site clinician should consider other possible causes for toxicity before discontinuing study medicines. When possible, concomitant medicines should be withheld first at the discretion of the site clinician if it is suspected that concomitant medicines are contributing to the toxicity. If after careful assessment and in the site investigator's judgment the event is at least possibly attributable to study drug(s), then the

suspected causative study drug(s) may be withheld; the participant should be permanently discontinued from study drugs if it is in their best interest.

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. Any participant for whom study drugs are temporarily held will be resumed on study medication as soon as possible.

12.4.1 Type and Duration of the Follow-up of Subjects after Adverse Events

Participants who experience adverse events that necessitate temporary or permanent discontinuation of study drugs will still be considered to be part of the study and will continue to be followed in the study in accordance with the study MOOP. If study drugs are permanently discontinued, further anti-tuberculosis therapy may be administered at the clinic staff's discretion according to local or National Tuberculosis Program guidelines.

12.5 Data and Safety Monitoring Board

The TBTC Data and Safety Monitoring Board (DSMB) will review the study protocol and oversee progress of the trial. The TBTC DSMB is comprised of a clinical TB expert, an epidemiologist with extensive experience with TB and HIV, and an internationally recognized clinical trialist/statistician, all of whom are not otherwise involved in the study. The DSMB will convene approximately annually, or more often as needed.

12.6 Interim Monitoring and Analyses by the Data and Safety Monitoring Board

The DSMB will have access to data and interim results and may recommend early closure of any experimental arm or the trial if, in their judgment, interim evidence is sufficiently strong that one of the trial interventions is clearly indicated or clearly contraindicated because of a net difference in efficacy, safety, or tolerability as defined by the corresponding primary endpoints for the trial, with a difference between arms that is statistically significant at the 0.1% level, according to the Haybittle-Peto statistical criterion for monitoring interim efficacy data. The analysis sequence described in section 13.5 will be followed for interim analysis. It is possible that arm 3 could be inferior to arm 1 (e.g., due to less adherence, drug interactions), but arm 2 not inferior to arm 1. If this is occurs, the study team will discuss implications with the DSMB.

13 STATISTICAL CONSIDERATIONS

13.1 Study Hypotheses

This study has two main hypotheses and consists of two comparisons:

A) In previously untreated individuals with drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by nine weeks of rifapentine, isoniazid, and moxifloxacin (2PHZM/2PHM), all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with the standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid) all given daily throughout.

B) In previously untreated individuals with drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide, and ethambutol followed by nine weeks of rifapentine plus isoniazid (2PHZE/2PH), all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with the standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

13.2 Study Outcome Measures

Primary Efficacy Endpoint:

- TB disease-free survival at twelve months after study treatment assignment

Primary Safety Endpoint:

- Proportion of participants with grade 3 or higher adverse events during study drug treatment

Secondary Efficacy Endpoints:

- TB disease-free survival at eighteen months after study treatment assignment
- Proportion of participants who are culture negative at eight weeks (solid and liquid media considered separately)
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated MGIT days to detection
- Sensitivity analyses assuming all losses to follow-up and non-tuberculosis deaths have an unfavorable outcome
- Sensitivity analyses assuming all losses to follow-up and non-tuberculosis deaths have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

13.3 Definition of primary outcome status

The primary analysis will be conducted using culture results from solid media and liquid media. Each participant will be classified into one of the following 3 outcome categories of Absence of Cure (Unfavorable Outcome), Cure (Favorable Outcome), or Not Assessable. An independent Endpoint Committee will be formed and be responsible for determining final outcome status. The primary efficacy endpoint will be assessed at 12 months after treatment assignment; a secondary efficacy endpoint will consider the follow-up period to be 18 months after treatment assignment.

Absence of Cure (Unfavorable Outcome) meeting any one or more of the following:

- Absence of bacteriological cure. A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17, that is culture positive for an *M. tuberculosis* strain that is indistinguishable from the initial isolate, and this is confirmed by a second sample that is culture positive for *M. tuberculosis*. A second confirmatory sample is required as a single positive sputum culture in isolation will not be considered absence of bacteriological cure.

- Participants who die from any cause during study treatment, except from violent or accidental cause (e.g. road traffic accident).

- Participants failing to complete treatment and not assessable at the end of the follow-up period.

- Participants who had a positive culture for *M. tuberculosis* when last seen, whether confirmed by a second sample or not, unless determined to have been re-infected

- Participants receiving any one or more of the following: a) extension of treatment beyond that permitted by the protocol; b) a re-start of treatment; c) a change in treatment for any reason except re-infection, pregnancy, or temporary drug challenge

Cure (Favorable Outcome) meeting any one of the following and not already classified as having an unfavorable outcome

- Participants with negative cultures at the end of the follow-up period

- Participants who at the end of the follow-up period are clinically without symptoms/signs of ongoing active TB and are unable to produce a sputum specimen

- Participants who at the end of the follow-up period are clinically without symptoms/signs of ongoing active TB and produce a sputum specimen that is contaminated without evidence of *M. tuberculosis*

Not Assessable (meeting any one or more of the following and not already classified as having an unfavorable outcome)

- Participants who completed assigned treatment and then default from follow-up, with their last culture being negative for *M. tuberculosis*

- Women who become pregnant during their assigned active treatment and stop their assigned treatment

- Participants who die during the follow-up phase (≥ 15 days after completion of study treatment)

- Participants who die from a violent (e.g. homicide) or accidental (e.g. road traffic) cause during their assigned active treatment. As above, suicide will be considered an unfavorable outcome

- Participants re-infected with a new strain of *M. tuberculosis,* demonstrated to be different from that identified at study entry through genotyping methods

13.4 Analysis Groups

There will be 4 analysis groups, as follows:

Intention-to-Treat (ITT)
Includes all enrolled participants who receive a treatment assignment.

Microbiologically Eligible
Includes the subset of Intention-to-Treat participants who, in addition, have culture confirmation of drug-susceptible tuberculosis at study entry. Participants classified as 'not assessable' will be considered to have an unfavorable outcome.

Assessable
Includes the subset of Microbiologically Eligible participants who, in addition, are not classified as 'not assessable'.

Adherent Per-Protocol
Includes the subset of Assessable participants who, in addition, complete assigned study treatment and follow-up.

13.5 Analysis Plan

13.5.1 Co-Primary efficacy analyses

There will be two co-primary efficacy analyses. One will consider the Microbiologically Eligible analysis population, and the other will consider the Assessable population. The assessable group is defined by exclusions that do not introduce selection bias. For each, the comparison of Regimen 1 (control regimen) versus Regimen 3 (2PHZM/2PHM) will be considered first, and, if non-inferiority criteria are met then the comparison of Regimen 1 versus Regimen 2 (2PHZE/2PH) will be considered. In each comparison, non-inferiority will be assessed by comparing the upper bound of a 95%, 2-sided confidence interval for the difference between the proportion of participants who are classified as having an unfavorable outcome on the control regimen (Regimen 1) and the intervention regimen to the predefined non-inferiority margin of 6.6%.

13.5.2 Primary safety analysis

There will be two comparisons: for comparison 1, Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH); for comparison 2, Regimen 1 (control regimen) will be compared against Regimen 3 (2PHZM/2PHM). The primary safety endpoint is proportion of participants with grade 3 or higher adverse events. The primary safety analysis will include the Intention-to-Treat group.

13.5.3 Secondary efficacy analyses

In secondary efficacy analyses, the primary efficacy endpoint will be assessed in the Adherent Per-Protocol analysis population, and secondary efficacy endpoints will be assessed for the Microbiologically Eligible, the Assessable, and the Adherent Per-Protocol analysis populations. Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH), and Regimen 1 will also be compared against Regimen 3 (2PHZM/2PHM). Secondary analyses will include those directed towards identification of clinical, microbiological, and/or radiological factors associated with favorable and unfavorable outcomes.

13.5.4 Secondary safety analyses

Tolerability will be assessed as discontinuation of the assigned treatment for a reason other than microbiological ineligibility. Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH) and Regimen 1 will also be compared against Regimen 3 (2PHZM/2PHM). This analysis will include the Intention-to-Treat group.

13.5.5 Secondary Efavirenz PK analysis

Efavirenz pharmacokinetics will be evaluated in the first 31 and then a total of 90 patients from each of two groups of participants randomized to treatment regimen 2 or 3 of the study and receiving efavirenz based ART. Participants receiving efavirenz will fall into one of two groups for the PK evaluation: 1) HIV-infected participants on efavirenz with virologic suppression at the time of study enrollment (EFV1), or 2) HIV-infected participants started on efavirenz after initiation but before or at week 8 of study tuberculosis drugs (EFV2). Individual-level measurements of efavirenz mid-dosing interval concentrations and estimates of efavirenz apparent oral clearance using a model-based post-hoc Bayesian approach will be performed. The efavirenz PK data will be judged acceptable if we have evidence that >80% of participants have estimated efavirenz mid-dosing interval concentrations **≥**1 mg/L.

13.6 Sample Size Considerations

The primary objective of the trial is to evaluate whether rifapentine containing regimens can produce outcomes at least as favorable as standard therapy, but with a shorter treatment course. Therefore, the trial is structured as a non-inferiority study.

Key assumptions:

- Primary endpoint rate: 15% absence of cure (unfavorable) in the standard regimen arm (Microbiologically Eligible population). This rate is based on observed results for the control arm (MITT analysis group) in two recently completed phase 3 clinical trials (27/161 [14%] in the

Rifaquin trial [Jindani et al., 2013] and 100/743 [13.5%] at 18 months post randomization and 114/679 [16.8%] at 24 months after the end of treatment in the Oflotub trial [Merle et al., 2013].)

- Margin to define inferiority: 6.6% (δ = 0.066)
- 95% confidence (type 1 error, α = 0.05). The sequential testing of regimen 3 and regimen 2 protects the type 1 error rate, as follows: If the statistical test for regimen 3 fails at 95% confidence, then conclude that both experimental regimens are not noninferior. If and only if regimen 3 is noninferior, then proceed to test regimen 2 at 95% confidence. A type 1 error occurs if either regimen is incorrectly deemed noninferior; the sequential approach limits the probability of this error to 5% overall.
- Power: 80% (type 2 error, β = 0.20) for primary analysis among Microbiologically Eligible subgroup, with power recalculated for the restriction to Assessable subgroup (see below)
- Proportion of enrolled patients who would be found to be late exclusions due to microbiological ineligibility – 12% (based on observed results in recent TBTC phase 2 studies)
- Proportion of enrolled patients who would be found to be 'not assessable': 12% (based on observed results in the Rifaquin trial [Jindani et al., 2013])

With 816 per arm, we expect 612 assessable. With the expected 15% unfavorable outcomes among those who are assessable, then with the same noninferiority margin and type 1 error rate, we have 90% power to test the primary hypotheses among the Assessable subgroup. The 6.6% margin to define inferiority (6.6%) takes into consideration the following issues: (1) the rates in historical trials of inpatient TB treatment for 6-month and 4-month regimens conducted by the British Medical Research Council support a difference in relapse up to 6% (East African/British Medical Research Council 1976, 1977, 1981; East and Central Africa/British Medical Research Council 1986; Singapore Tuberculosis Service/British Medical Research Council 1986; Nunn and Crook 2013); (2) recent trials in contemporary outpatient populations suggest a higher baseline proportion (15%) of unfavorable outcomes likely to be observed based on phase 3 trials and definitions; (3) the investigators in this trial and others perceive that the benefits of reducing treatment duration to 3 or 4 months would have advantages not outweighed by a possible increase in the relapse rate of up to 6%; and (4) the 6.6% margin does not imply that the experimental regimen may result in as much as 6.6% more unfavorable outcomes, but rather, for a fixed design, the maximum difference consistent with a non-inferior conclusion decreases as the proportion of unfavorable outcomes in the control arm increases.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Study staff will be trained in Good Clinical Practice and in performance of study procedures. The sponsor or the sponsor's delegate will conduct a meeting with each site prior to the trial opening at the site in order to ensure that everything is in place for the trial to start, the study file contains all the essential documents, and study staff understand procedures and their roles and responsibilities. The study will be conducted in accordance with the protocol and study-specific procedures manuals.

14.1 Local quality control

Each site will have a written plan for local quality control including data quality management.

14.2 External monitoring

External monitoring will be conducted to ensure the safety and conduct of this study, which involves patients with a serious illness (i.e. tuberculosis), uses an investigational drug treatment, will take place in multiple international sites, and may be used to modify the license for rifapentine and moxifloxacin.

External monitoring will be conducted periodically by a contract research organization (CRO). Direct access to data at each site will be required for the purposes of monitoring and audit, and this will be made explicit in the consent form. Local investigators and their institutions will provide direct access to source documents and data for trial-related monitoring, audit, and regulatory inspections, in the clinic, the pharmacy, and the mycobacteriology laboratory.

External monitoring will be conducted according to a written manual of procedures. Monitoring will focus on ensuring that the following study activities are conducted per the protocol and associated documents: consent procedures, enrollment, dispensing of trial medications, correct implementation of treatment and follow-up procedures, accurate recording and reporting of adverse events and appropriate reporting of adverse events, correct implementation and reporting of mycobacteriology laboratory testing, accurate completion of case report forms, and timely and accurate data entry. Additional activities or study elements may be monitored as needed.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

This study will be conducted in conformity with the ethical standards set out in the latest version of the Declaration of Helsinki.

15.1 Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents by an appropriate institutional ethics committee (IEC) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use.

15.2 Informed Consent Process

Adults: Only individuals who provide written informed consent will be enrolled in this study. Written informed consent is required before any study-specific procedures are performed. Potential participants will have the conditions of the study explained to them, including potential harms and benefits, the nature and timing of study procedures, alternatives to study participation, that study participation is voluntary, that a decision to not participate in the study will not affect the quality of their future medical care, and that they may withdraw from participation at any time. The information in the Informed Consent document will be translated into relevant local languages. Literate individuals will be provided with a language-appropriate document to read; illiterate individuals (i.e. individuals who speak and understand, but do not read and write, the language in which the consent discussion is conducted) will have the contents of the document explained to them by a trained study staff member; such individuals can be enrolled by 'making their mark' on the consent document. Potential participants will have the opportunity to ask questions of the site investigator or delegate, and to discuss participation with their family and/or friends or think about the study prior to deciding whether or not to participate. A copy of the signed informed consent document will be given to the participant for his/her records.

Children: For potential participants under 18 years of age, the assent of the child as well as the written informed consent of the child's legal guardian will be required for enrollment in this study. The child will receive, in language appropriate to the age and maturity of the child, an explanation of the research procedures; a description of the risks, discomforts, or inconveniences that the child might experience; and assurance that the child can withdraw from the study at any time. The assent process will be conducted by a study staff member who is experienced in consent and assent procedures, and in accordance with IRB/IEC requirements. All study participants age < 18 years will provide oral or written assent and written consent by the participant's legal guardian, in accordance with national and/or local IRB/IEC requirements.

15.3 Subject Confidentiality

All records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available without sufficient de-identification procedures.

Participant names will not be supplied to the sponsor. All study documents and forms will be identified by a code only. All paper study records will be stored in a locked office and electronic study records will be stored on password-protected computers; only designated trained study staff will have access to study

records. Transmission of electronic records to the sponsor will occur through a web-based data entry application that conforms to the Federal Information Security Management Act, or using a secure CDC File Transfer Protocol (FTP).

The study monitors, other authorized representatives of the sponsor, and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records, primary laboratory data, and pharmacy records for study participants; this information will be provided to participants during the Informed Consent process. The clinical study site will permit access to such records.

15.4 Study Discontinuation

The Data and Safety Monitoring Board may recommend study termination or the termination of a study arm. In addition, after consultation with involved parties, the Sponsor has the right to close the study and the right to close a study site. If the study is closed, all involved ethics committees should be notified. If a site is closed, then the local IEC/IRB and the CDC IRB should be notified. In the event that the study is discontinued or a study site is closed, participants will undergo an early termination study visit. Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event. Participants who were receiving study treatment at the time of early termination will be referred to local sources for tuberculosis care.

16 DATA HANDLING AND RECORD KEEPING

ICH-GCP requirements for data documentation will be followed.

Data handling and record keeping will be performed in accordance with a study MOOP. Briefly, information from source documents will be recorded on study-specific case report forms, which will be entered into the study electronic information system. The study electronic information system will be programmed to maintain an audit trail, perform consistency checks, and generate reports of missing/inconsistent data. After study completion, de-identified data that can be legally released to the public may be released through a public-use data set after the data are evaluated for quality and confidentiality and shared with any partners, per CDC's policy on data sharing.

The study file and source documents should be retained until the sponsor gives notification.

17 PUBLICATIONS AND DISSEMINATION OF STUDY RESULTS

The study lead investigators and the TBTC Publications and Presentations committee must approve the use of any coded study data for the purposes of publication or presentation in advance. Any proposal for collection of additional data or analysis of study data must be agreed to in advance by the study lead investigators and the protocol team. The preparation of and authorship of publications arising from this study will be in accordance with the TBTC Bylaws.

These criteria will not apply to public-use data that have been made available in accordance with CDC's policy on data sharing. Persons who use publicly available data will be asked to acknowledge the TBTC and the S31 Protocol Team.

Updates on the progress of the trial will be presented at the twice-yearly TBTC meetings. Additional dissemination of results will be through the press, national governments at meetings and international organizations at conferences. Overall (aggregate) study results will be shared with study participants through mechanisms and materials reviewed and approved by the TBTC Community Research Advisors Group.

18 LITERATURE REFERENCES

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SUPPLEMENTS/APPENDICES

Appendix A: Schedule of Procedures/Evaluations

Visit Window		Up to 7 days after screen	+/- three (3) days						+/- seven (7) days					**Possible Poor Treatment Response**	**Post Early Termination Visit[h]**
Visit	**Screen**	**Baseline**	**WK 2**	**WK 4**	**WK 8**	**WK 12**	**WK 17**	**WK 22**	**WK 26**	**MO 9**	**MO 12**	**MO 15**	**MO 18**		
Informed Consent	X														
Inclusion/Exclusion	X	X													
Demographics	X														
Contact information	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X														
Symptoms		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X						X
Interval medical history										X	X	X	X	X	
Height	X														
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chest radiograph	X						X[f]		X[f]					X	
Visual tests		X		X											
HIV test	X														
CD4 count (if HIV-pos)	X[a]														
HIV Viral load (if HIV-pos)	X[a]														
Pregnancy testing	X														
Randomization		X													
Sputum for smear and culture[b]	X	X	X	X	X	X	XX	XX	XX	XX	XX[c]	XX	XX[c]	XXX	
Sputum for rapid molecular test, if available at site	X														
Storage of Mtb bacterial isolate	X	X					X	X	X	X	X	X	X	X	

Diabetes screen[d]	X														
ALT	X		X	X	X	X	X	X							X
Bilirubin	X		X	X	X	X	X	X							X
Creatinine	X		X	X	X	X	X	X							X
Hemoglobin	X		X	X	X	X	X	X							X
WBC with differential	X		X	X	X	X	X	X							X
Platelets	X		X	X	X	X	X	X							X
Albumin	X														
Potassium	X														
PK sampling for TB drugs[g]			Obtain within this interval												
Blood sampling for pharmacogenomics testing		Obtain any time after enrollment													
EFV1: Plasma for EFV PK	Once at screening or baseline			X	X		X								
EFV1: HIV viral load	Required per eligibility criteria				X		X								
EFV2: Plasma for EFV PK [e]				Obtain at about 4 weeks after starting EFV AND at about 8 weeks after starting EFV				X							
EFV2: HIV viral load [e]	Required per eligibility criteria			Obtain once at about 8 weeks after starting EFV				X							
Sputum, blood, and urine collections for research (consenting participant only)		X	X	X	X		X[f]		X[f]					X	
Contact central study clinician														X	
Visit	**Screen**	**Baseline**	**WK 2**	**WK 4**	**WK 8**	**WK 12**	**WK 17**	**WK 22**	**WK 26**	**MO 9**	**MO 12**	**MO 15**	**MO 18**	**Possible Poor Treatment Response**	**Post Early Termination Visit[h]**
Visit Window		Up to 7 days after screen	+/- three (3) days						+/- seven (7) days						

NOTES

a Unless results of a test performed at or within 30 days prior to screening are available.

b All sputa should be sent to the designated study laboratory with the exception of the screening specimen, which may be evaluated at any locally acceptable laboratory. If a screening specimen has been found to be smear or culture positive at a non-study laboratory, then either store the isolate if culture positive from the non-study laboratory or get an additional specimen for culture and storage of isolate. Two specimens (i.e. one at screening and one at baseline) are required prior to initiation of study treatment. At least two (2) sputa should be obtained at each of weeks 17, 22, 26 and at each of months 9, 12, 15, and 18.

c If both of the month 12 sputa or both of the month 18 sputa are contaminated, then the participant should be asked to provide at least two (2) additional sputa as soon as possible after contamination is recognized.

d Hemoglobin A1C is the preferred test. If such testing is not available, then fasting or random blood glucose can be measured.

e see Section 8.3.10.1.2 for recommended schedule based on timing of initiation of efavirenz-based ART and coinciding with scheduled study visits

f To be obtained at the end-of-study treatment visit (i.e. either week 17 or week 26).

g See MOOP for guidance on selection of participants for this sampling

h See Section 9.9 “Post Early Termination Visit (Visit after Early Termination) for Participants Terminating Before Completion of their Assigned Study Treatment.” This visit occurs approximately 14 days after stopping study drugs.

Appendix B: Abbreviations

Abbreviation	Full-length identification
ACTG	AIDS Clinical Trials Group
AE	Adverse event
ALT	Alanine aminotransferase (alternative term is serum glutamic-pyruvic transaminase [SGPT])
ART	Anti-retroviral therapy
AUC	Area under the concentration-time curve
CDC	Centers for Disease Control and Prevention (United States)
CFR	Code of Federal Regulations (United States)
CRO	Contract research organization
DOT	Directly observed therapy
DSMB	Data and Safety Monitoring Board
E	Ethambutol
EFV	Efavirenz
FDA	Food and Drug Administration (United States)
G	Gatifloxacin
GCP	Good Clinical Practice
H	Isoniazid
ICH	International Conference on Harmonisation
INH	Isoniazid
IEC	Institutional ethics committee
IRB	Institutional review board
ITT	Intention-to-treat
M	Moxifloxacin
MDR	Multidrug-resistant
MGIT	Mycobacterial Growth Indicator Tube

MIC	Minimum inhibitory concentration
MITT	Modified intention-to-treat
MOOP	Manual of Operating Procedures
MTB	Mycobacterium tuberculosis
P	Rifapentine
PD	Pharmacodynamics
PK	Pharmacokinetic
PP	Per-protocol
PZA	Pyrazinamide
R	Rifampin (alternative term is rifampicin)
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
TBTC	Tuberculosis Trials Consortium
WHO	World Health Organization
XDR	Extensively drug resistant
Z	Pyrazinamide

Date: May 14, 2015
Re: Protocol Amendment for: "Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: A randomized, open-label, controlled phase 3 clinical trial" from v1.1 to v2.0
To: CDC IRB
From: Stefan Goldberg

We wish to amend the current protocol version to include the changes described below. Tracked and clean versions of the consent, assent, and protocol are attached. Thank you in advance for your consideration of these changes, which, from our perspective, will enhance successful implementation of this study.

CONSENT AND PERMISSION FORM CHANGES

- The new version is 2.0 and new date is 14 May 2015

- The footer has been revised to now include the ACTG protocol number, A5349. The footer now reads "Flesch-Kincaid Grade Level 7.5 / S31/A5349, Version 2.0, 14 May 2015." The reading level increased from 7.2 to 7.5.

- To the section, "your participation is voluntary," we have added, "You will be informed of any new findings during the course of the study that may affect your willingness to continue participation. After the study is completed, you will be informed of the study results."

- To the section, "why is this study being done," we have added, "The study will also see what side effects are caused by taking rifapentine and moxifloxacin for 4 months."

- To the section, "who has reviewed this study," we have added, "The role of an IRB is to protect the rights, safety and wellbeing of people in a clinical trial."

- To the section, "other visits during study treatment," we have added a description of the staged enrollment of persons co-infected with HIV, related to their efavirenz-treatment status, to help clarify this part of the protocol plan. We add names for the two evaluation stages: EFV1 and EFV2. We also add a statement that blood drawn for efavirenz evaluation will be sent to a laboratory in the U.S.

- Below the description of the efavirenz plan we add a term to start the next section, "Sparse PK," which is defined after the first sentence. We deleted the limitation of sparse PK testing to research treatment groups 2 and 3. As stated in protocol section 8.3.9.1, individuals not undergoing intensive PK sampling will have sparse PK sampling regardless of assigned study arm. We deleted blood volume from this section because it is specified in a table below. We deleted South Africa from the statement of where these samples will be sent for testing. (On the submission date of this amendment, the selection process still is underway for the lab that will do these tests: remaining candidate labs are in the U.S. and Europe.) Detailed timing of these blood draws has been deleted, as unnecessarily technical for the consent form and to

allow flexibility in the manual of operating procedures (MOOP) to specify ideal and acceptable time ranges.

- The table of events has been modified for clarity and to match minor changes listed below for the protocol.

- In the section, "could your participation end early," we have inserted "study treatment and/or" in the statement about why your participation in the study might be stopped early, to clarify that study participation is not synonymous with study treatment.

- To the section, "what happens if you are injured because you took part in this study," we have added, "If you have a research-related injury or if you experience an adverse reaction, you should contact your study doctor. See the section "Contact Information" for phone numbers and additional information."

- We have added an opt-in or opt-out section for genetic testing (pharmacogenomics only): "Collection of a blood sample for genetic testing," to give participants these options. (protocol section 8.3.11).

- We have modified the heading and text of the additional biomarker specimen signature block to emphasize collection in addition to storage of these specimens for additional research studies. We have deleted the sentence about genetic testing in these specimens, since genetic testing is not planned for these specimens (protocol section 8.3.13). We also added an instruction to sites that this section is to be included in their consent form if they are participating in this biomarker specimen collection activity.

ASSENT FORM CHANGES

- The new version is 2.0 and new date is 14 May 2015

- The footer has been revised to now include the ACTG protocol number, A5349. The header now reads "Flesch-Kincaid Grade Level 6.6 / S31/A5349, Version 2.0, May 14, 2015." The reading level increased from 6.4 to 6.6.

- The section on study procedures has been modified for clarity:

    - We added the purpose of drawing blood: "to check your liver, kidney, and blood counts."
    - We added the purpose of sputum collection: "The sputum will be tested for the TB germ."
    - We moved the end-of-treatment chest X-ray line to the subsection about procedures that follow completion of treatment.
    - We added a line describing blood collection for PK assessments
    - We added a statement about additional blood collection for efavirenz testing in persons with HIV infection.
    - We added a statement about optional additional blood collections for biomarker studies.

- To the section, “why should I take part in this study,” we added the sentence, “There are no direct benefits to you for taking part in this study.”

- We added a section, “what if I do not want to take part in this study?”

    - To this section we added that the participant and parent or guardian would be informed of new findings that might affect willingness to continue participating.
    - We added a statement that the participant and parent or guardian would be informed of study results after completion of the study.

- To the section, “what if I have some questions or change my mind,” we have added “If you choose to take part in this study and then change your mind, your regular medical care will not be affected. You can get TB treatment through the Health Department or your regular doctor” and moved “You may stop being in the study at any time, after talking about it with your parent or guardian.”

- In the section, “how do I sign up,” we have changed the statement the reference to parental “consent” to a reference to a “permission form” needing to be signed by the parent or guardian.

PROTOCOL CHANGES

ADMINSTRATIVE CHANGES

- The new version is 2.0 and new date is 14 May 2015

- The header has been revised to now include the ACTG protocol number, A5349. The header now reads “TBTC S31/A5349: Rifapentine-containing tuberculosis treatment shortening regimens”

- The study has been registered on Clinicaltrials.gov as NCT02410772; this identifier has been placed on the protocol cover page.

- The protocol team member listing has been updated. Suria Yesmin (Social & Scientific Systems, Inc, Silver Spring, MD), Sachiko Miyahara (Harvard T.H. Chan School of Public Health, Boston, MA), and Lisa Wolf (Johns Hopkins University School of Medicine, Baltimore, MD) have been added at the request of ACTG leadership. Statistician Michael Chen, as well as Pei-Jean Feng and Erin Sizemore from CDC/TBTC have been added; Patricia Bessler from CDC/TBTC is no longer serving on the protocol team and her name has been removed.

SCIENTIFIC AND PROCEDURAL CHANGES

- Section 7.2: the text has been revised to reflect that, for HIV, there will be two strata (HIV-positive and HIV-negative) rather than three strata. This change reduces the risk of assigning participants to strata that would be too small to analyze usefully.

- Section 8.1.7: the timing of end-of-treatment chest radiographs has been corrected to reflect that the end-of-treatment chest radiograph will be performed at the completion of assigned treatment, which may be either at week 17 (investigational arms) or week 26 (control arm). The rationale is that the end-of-treatment chest radiograph is a clinically important tool for documenting extent of residual radiographic changes at the time of treatment completion, as this information can be used to help interpret the potential significance of future chest symptoms/signs, should they occur. Sections 9.4 and 9.6, weeks 17 and 26 visit task lists, and the event schedule, appendix A, also has been updated with this change.

- Section 8.3.9.3 has been revised to increase the volume of blood that will be collected at each of the tuberculosis drug sparse PK blood draws. Formerly the volume was 6 ml, but the volume has been increased to 10 ml to ensure that the volume of serum is appropriate for two or more aliquots.

- Section 8.3.10.1 has been revised to decrease the volume of blood that will be collected at each of the efavirenz PK blood draws. Formerly the volume was 8 ml, but the volume has been reduced to 6 ml after discussions with the laboratory team.

- Section 8.3.10.1 "Efavirenz Sampling Strategy" and the Appendix A Schedule of Events have been revised in order to assess the potential significance of low-positive viral load results. For HIV-infected participants undergoing protocol-specified HIV viral load testing while on study treatment and efavirenz (e.g. at weeks 8 and 17), if a viral load result is greater than 200 copies/mL then approximately 2 to 4 weeks after that test, blood should be drawn and a repeat HIV viral load test performed.

- Section 8.3.11 Pharmacogenomic testing: Specific consent for genetic testing is added, with a new section added to the consent form for participants to opt in or opt out of this testing, without affecting participation in the treatment study.

- A new section entitled 'Participants with a possible poor treatment response' has been added (Section 8.8) to clarify the scenarios under which a participant should be considered to have a possible poor treatment response and therefore undergo assessment.

- Sections 8.6 through 8.8 and 8.9.3 have been revised to now remove the requirement that the central study clinician be contacted prior to on-site clinical decision-making. This change also has been made in the section 2.2, "rationale," under "rationale for an open-label study:" clarifying that the central study clinician is "available to advise on protocol procedural issues," and in Section 10.6 "Criteria for Discontinuation of Study Drugs."

- Section 9.8 has been revised to clarify and present in itemized fashion the study assessments that should be performed for participants deemed by the site investigator as having a possible poor treatment response. This information has also been added to the Appendix A Schedule of Events.

- Section 9.11 “Unscheduled Visit” has been expanded and revised. Specifically, the section has been expanded now to two sections, named “Missed Visit” (9.11) and “Unscheduled Visit” (9.12). Definitions of missed and unscheduled are provided.

- Section 12.3.2 “Reporting Procedures” the following information has been added: “The DAIDS Medical Officer will receive a copy of the IND safety reports that TBTC submits to the FDA”.

- Section 13.5.3 “Secondary Efficacy Analyses” has been updated to add secondary analyses directed towards identification of clinical, microbiological, and/or radiological factors associated with favorable and unfavorable treatment outcomes.

CLARIFICATIONS / CHANGES TO INCREASE CLARITY AND UNDERSTANDING

- Section 3 Descriptions of study drugs. We add specification that the study drugs are “commercially available as brand or generic.”

- Section 3.3 Moxifloxacin. We deleted the brand name, Avelox®, because moxifloxacin is available as a generic product and the protocol does not specify the use of Bayer’s brand name product.

- Section 6.1: Subject Inclusion Criteria. Eligibility criteria have not been changed. However, in two instances additional information has been added for clarity and for consistency with other sections of the protocol:
    - Inclusion Criterion D has been clarified to indicate that a non-hormonal intrauterine device is considered (for study purposes) an adequate means of contraception

    - Inclusion Criterion F has been revised to now include the information about staged enrollment that previously was present only later on in the protocol (Section 8.3.10). Our perspective is that adding this detail directly to the Inclusion Criterion enhances clarity and therefore will help to ensure correct implementation of subject selection and enrollment procedures.

    - Inclusion criterion H has been modified to define childbearing potential more specifically, to improve guidance on the pregnancy test requirement.

- Section 6.2 Criteria for Exclusion.
    - Exclusion criterion I: We have deleted the statement that individuals currently on or planning to start efavirenz-based antiretroviral treatment are included, as redundant and potentially confusing, especially since we have added efavirenz-related clarification to inclusion criterion F, as described above.

- Section 8.2 Concomitant Medications/Treatments has been modified to describe the indication and use of non-hormonal contraception more clearly.

- Section 8.3.9 Pharmacokinetic sampling: Tuberculosis drugs

- 8.3.9.1 Overview: Europe has been added and South Africa deleted as possible locations for testing of blood specimens for concentrations of TB drugs and their metabolites.
- 8.3.9.2 Timing of sparse PK sampling: "for rifapentine and moxifloxacin" has been deleted since this testing will be done regardless of treatment arm, as stated in section 8.3.9.1 Overview.
- 8.3.9.3 Schedule of sparse PK blood samples: "for rifapentine and moxifloxacin" has been deleted. Blood volume is corrected to 10 ml for each specimen, from 6. Time span for the two or three blood draws has been corrected to a 9 hour period from 8. Specific timing details for the blood draws have been deleted in order to allow the MOOP to specify ideal and acceptable time ranges.

- Section 8.3.10 has been revised to clarify that participants randomized to the 2HRZE/4HR control arm will not have efavirenz PK evaluations.

- In Section 8.3.10, what previously had been called 'Group 1' and 'Group 2' has been revised to 'EFV1' and 'EVF2' for clarity. These terms also are applied in the event schedule, Appendix A, and in the consent form, to match usage across these documents.

- The protocol Appendix A Schedule of Events has been corrected in order to harmonize with protocol text. Corrections to Appendix A are as follows:

  - Addition of efavirenz blood sampling at the week 4 visit for individuals in 'EFV1' in accordance with the information already contained in the protocol text in Section 8.3.10.
  - For clarity, we have re-formatted the Appendix A information for EFV1 and EFV2 (efavirenz PK procedures)
  - For clarity, we have added a column that indicates procedures and evaluations to be performed for participants with possible poor treatment response
  - For clarity we have renamed the final column "post early termination visit," from "early termination during treatment," matching the protocol sections 8.9.1 and 9.9 to clarify that the procedures in this column are to be done approximately 2 weeks <u>after</u> the last study dose, testing for late toxicity, rather than at the time of the last study dose.

- Section 8.7 "Participants with a positive sputum smear or culture at or after week 17:" The word, "alone," is inserted in the sentence, "Sputum smear results alone should not be used for clinical decision-making," to acknowledge that smear results usually do contribute, while emphasizing that they should not be the only guiding factor.

- Section 8.9 "Management of a participant who is discontinued from study treatment" has an added introductory sentence to clarify that "for any participant who is discontinued from study treatment, the assessments listed in Section 9.9 "Post Early Termination Visit" should be performed." "Post early termination visit" is used in the protocol now to indicate the visit that occurs 14 days after early termination of study drugs, to evaluate for delayed toxicity.

- Section 8.3.13 “Samples for analyses to identify potential biomarkers” has been modified to specify that these samples are collected only “at participating sites.” An ACTG substudy protocol, analogous to TBTC Study 36A (CDC IRB #6556), has been added for reference in this section: Biobank for Surrogate Marker Research for TB (A5302). With A5302, selected ACTG sites are contributing specimens to the same multi-consortium repository to which selected TBTC sites are contributing with Study 36A.

- Section 9 “Study Schedule:” the procedure list for each visit after screening has been modified to add “at least” to the number of specified sputum specimens to collect, in order not to limit site staff from collecting more specimens if clinically indicated.

- Section 9.9 is renamed, from “Early Termination Visit” to “Post Early Termination Visit” in order better to match the instruction in the section that this visit occurs “approximately 14 days after stopping study drugs.” This label is updated elsewhere in the protocol too, especially in the event schedule, Appendix A. Review of contact information is added as a task for this visit and sputum collection and chest radiograph are deleted, as unnecessary for study purposes at this time point. A sentence is added to help distinguish procedures indicated at a visit at which possible poor treatment response is determined, from those indicated at this visit.

- Section 9.10 “Early Termination Visit for Participants Terminating After Completion of their Assigned Study Treatment” has been renamed “Early Termination Visit for Participants Stopping Study Participation After …” This change and minor modifications in the first paragraph of the section are intended to maintain opportunities to assess crucial study questions to the extent that participants are willing to contribute.

- Section 9.11 “Early/Late Visit” is deleted, as redundant after the minor procedural changes described above for Sections 9.11 Missed Visit and 9.12 Unscheduled Visit.

- Section 12.2.1 “Adverse Events and Serious Adverse Events:” A time limit for ascertainment of AEs and SAEs is added to specify “up to and including Week 26.” This clarifies the ascertainment time frame for site staff with a consistent stopping time across treatment arms.

- Section 12.3.2 “Reporting Procedures” has been clarified and corrected to add relatedness to the definition of suspected unexpected serious adverse reaction (SUSAR). The correct information is “A SUSAR is defined as an adverse reaction that is unexpected (i.e. not consistent with the applicable product information) AND also meets the definition of a serious adverse event AND also may reasonably be regarded as caused by or associated with the administration of the study drug regimen (i.e. at least ‘possibly associated’ with the study drug regimen).”

- Section 12.4: The word “suspected” has been inserted in the last sentence of the first paragraph before “causative study drug(s) may be withheld,” to clarify that withholding can be done without proof of attribution.

MINOR CORRECTIONS AND EDITS

- The Table of Contents has been updated to reflect the changes described above

- Minor edits have been made to improve the accuracy of the information presented in the Background section on ‘Moxifloxacin for TB Treatment.’

- Section 9.3 has been corrected to remove from the Week 12 visit the collection of specimens for frozen storage, to match section 8.3.13 and the event schedule, Appendix A.

- Section 12.2: We have corrected the name of the toxicity tables that will be used from ‘Common Toxicity Criteria’ (the name formerly used) to now ‘Common Terminology Criteria for Adverse Events’.

- Several typing, spelling, and line spacing errors have been corrected

TITLE

Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: A randomized, open-label, controlled phase 3 clinical trial

Funding Agencies:
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Study Chairs:
Payam Nahid, M.D., M.P.H.
Susan Dorman, M.D.

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Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR, and applicable site-specific regulatory requirements.

Table of Contents

Protocol Summary

Title: Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: a randomized, open-label, controlled, phase 3 clinical trial

Hypotheses:

A) Seventeen (17) week rifapentine-based regimen

In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine (P), isoniazid (H), pyrazinamide (Z) and ethambutol (E) followed by nine weeks of rifapentine plus isoniazid, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin (R), isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

B) Seventeen (17) week rifapentine- plus moxifloxacin-containing regimen

In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide and moxifloxacin (M), followed by nine weeks of rifapentine, isoniazid, and moxifloxacin, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

Phase: 3

Design: This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.

Population: Patients with newly diagnosed, previously untreated pulmonary tuberculosis.

Number of Sites: Multiple international sites, primarily sites of the Tuberculosis Trials Consortium and the AIDS Clinical Trials Group.

Study Duration: Duration per participant is approximately 18 months.

Description of Agent or Intervention: After written informed consent, participants will be randomly assigned to receive one of the following oral regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

Objectives:

Primary:

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

Secondary:

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and store biospecimens from consenting participants for the purpose of future research on discovery and validation of TB biomarkers

- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, culture status at completion of eight weeks of treatment, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among patients with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine

Endpoints:

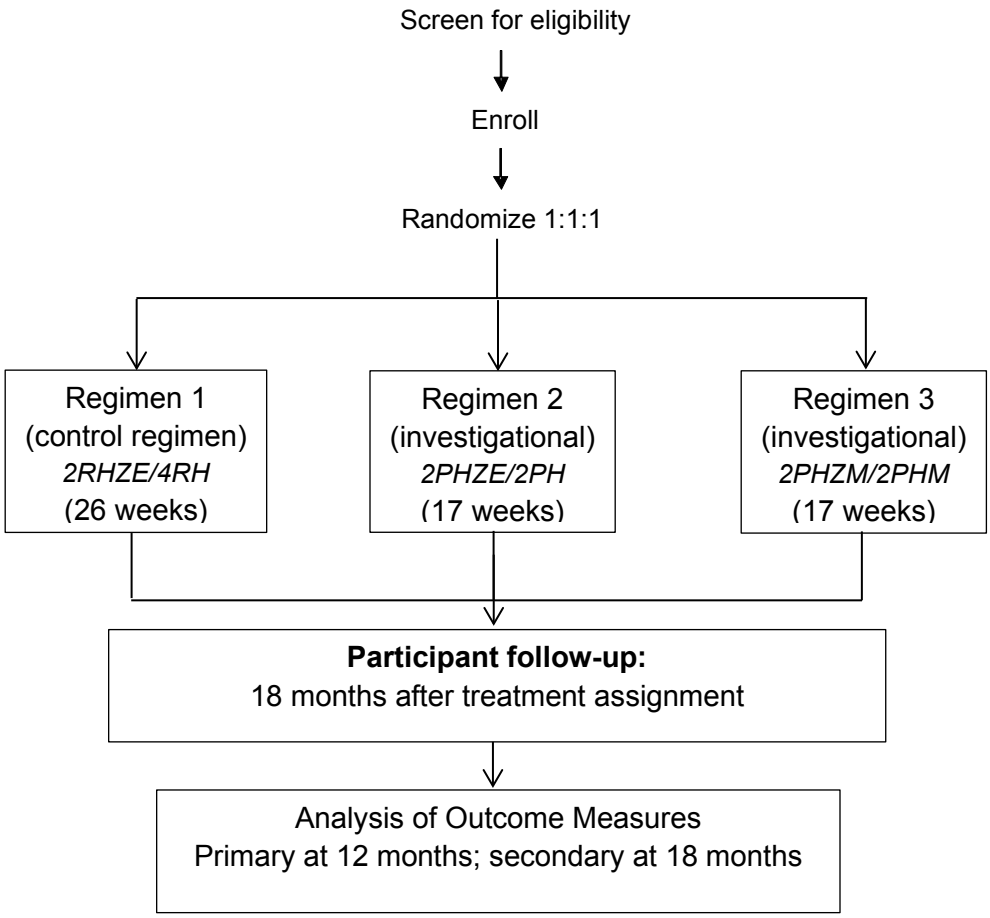
Primary Endpoints:

- Efficacy: TB disease-free survival at twelve months after study treatment assignment.
- Safety: Proportion of participants with grade 3 or higher adverse events during study drug treatment

Secondary Endpoints:

- TB disease-free survival at eighteen months after study treatment assignment
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection
- Proportion of participants who are culture negative at completion of eight weeks of treatment (solid and liquid media considered separately)
- Sensitivity analyses assuming all participants classified as ‘not assessable’ have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

Schematic of Study Design:



1 KEY ROLES

Funding Agencies:
U.S. Centers for Disease Control and Prevention through the Tuberculosis Trials Consortium;
U.S. National Institute of Allergy and Infectious Diseases of the National Institutes of Health through the AIDS Clinical Trials Group

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Protocol Chairs:
Payam Nahid, M.D., M.P.H.
University of California, San Francisco
1001 Potrero Ave, 5K1
San Francisco, CA 94110
Phone: 415-206-5464
Email: pnahid@ucsf.edu

Susan E. Dorman, M.D.
Johns Hopkins University School of Medicine
1550 Orleans Street, CRB2, 1M-12
Baltimore, Maryland, USA 21231
Phone 410-502-2717
Email: dsusan1@jhmi.edu

Project Officer:
Stefan Goldberg, M.D.
US Centers for Disease Control and Prevention
1600 Clifton Road, MS E-10
Atlanta, GA, USA 30333
Phone: 404-639-5339
Email: ssg3@cdc.gov

Central Study Clinician:
TB clinician(s) external to the protocol team, TBD

Protocol Team

Name	Institution
Janet Andersen	Harvard School of Public Health, Boston, Massachusetts, USA
Patricia Bessler	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Richard Chaisson	Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Kwok-Chiu Chang	TB and Chest Service of Hong Kong, China
Mark Cotton	Stellenbosch University, Cape Town, South Africa
Dalene von Delft	Community Research Advisory Group, Cape Town, South Africa
Kelly Dooley	Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Melissa Engle	University of Texas Health Science Center, San Antonio, Texas, USA
Courtney Fletcher	University of Nebraska Medical Center, Omaha, Nebraska, USA
Phan Ha	National TB Program, Hanoi, Vietnam
Charles M Heilig	US Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Daniel Johnson	Division of AIDS, National Institutes of Health, Bethesda, Maryland, USA
John L. Johnson	Case Western Reserve University, Cleveland, Ohio, USA
Marilyn Maroni	Sanofi, Paris, France
Cynthia Merrifield	University of California, San Francisco, San Francisco, California, USA
José M. Miro	Hospital Clinic Universitari, Barcelona, Spain
Nguyen Viet Nhung	National TB Program, Hanoi, Vietnam
April Pettit	Vanderbilt University, Nashville, Tennessee, USA
Anthony Podany	University of Nebraska Medical Center, Omaha, Nebraska, USA
Kathleen Robergeau	Westat, Inc., Rockville, Maryland, USA
Wadzanai Samaneka	Parirenyatwa Clinical Research Site, Harare, Zimbabwe
Susan Swindells	University of Nebraska Medical Center, Omaha, Nebraska, USA
Andrew Vernon	Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Marc Weiner	Audie L. Murphy Veterans Affairs Medical Center / University of Texas Health Science Center, San Antonio, Texas, USA

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Tuberculosis as a global health problem
Tuberculosis (TB) is one of the most important global health problems. According to recent estimates from the World Health Organization (WHO), 8.6 million new cases and 1.3 million deaths from TB occurred in 2012 (World Health Organization 2013). The vast majority of TB cases and TB deaths are in developing countries. The spread of HIV has fueled the TB epidemic, and TB is the leading cause of death among patients infected with HIV (Corbett et al., 2003). TB predominantly affects young adults in their most productive years of life and has substantial impact on economic development.

Need for new treatment regimens for tuberculosis
Although effective therapy for drug susceptible *Mycobacterium tuberculosis* is available, TB continues to cause significant morbidity and mortality worldwide, and rates of multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB cases are on the rise. A major obstacle to the control of TB is poor adherence with lengthy (at minimum 6 months) and complicated treatment regimens. Incomplete TB treatment can lead to increased morbidity and mortality, prolonged infectiousness and transmission, and the development of drug resistance. The use of directly observed therapy (DOT) can improve patient adherence and reduce the emergence of resistant microorganisms, but is logistically difficult and expensive to implement (McDonald et al., 1982). The development of new treatment strategies with more potent antimycobacterial activity could lead to shorter and better tolerated regimens. A TB treatment regimen that allowed a decrease in treatment duration would potentially have important public health implications by facilitating DOT, increasing cure rates, potentially reducing transmission and preventing emergence of MDR TB. It is estimated that improved regimens that shorten treatment duration for drug-susceptible strains and are efficacious against resistant strains could reduce the incidence of TB by up to 27% by 2050 and reduce deaths by 25% from current global numbers of incident cases and deaths per year (Abu-Raddad et al, 2009).

Current standard treatment for pulmonary tuberculosis
Modern short course treatment for pulmonary tuberculosis is comprised of two treatment phases. The intensive phase is the initial 8 weeks of treatment, and typically is comprised of isoniazid, rifampin, pyrazinamide, and ethambutol. Continuation phase follows intensive phase, and continuation phase typically is comprised of isoniazid plus rifampin for an additional 18 weeks, to complete a total of 26 weeks (6 months) of treatment.

Rifamycins in tuberculosis treatment
Rifamycins are the key drugs in modern short-course TB chemotherapy of 6 months duration. Rifamycins, including rifampin and rifapentine, have concentration-dependent activity against *M. tuberculosis.* Rifamycins are considered critical for sterilization, that is, prevention of relapse after cessation of TB treatment. For rifapentine, the minimum inhibitory concentration (MIC)50 and MIC90 are one- to two-fold dilutions lower than those of rifampin (for the 7H10 agar system, rifapentine's MIC50 and MIC90 are 0.125 and 0.25 mg/L, compared with 0.5 and 1.0 mg/L for rifampin) (Bemer-Melchior et al., 2000). In addition, rifapentine's half-life (t1/2) is five times longer than that of rifampin (14-18 hours vs. 2-5 hours).

Preclinical studies of rifapentine

Murine model of tuberculosis treatment
The murine model of TB has been used for more than 50 years for the development and evaluation of new antituberculosis drugs and regimens (Veziris et al., 2005). Importantly, the mouse model of TB treatment has been shown to recapitulate human TB treatment with regard to treatment-shortening effects of rifampin and pyrazinamide. In the mouse model, the standard 6-month rifampin plus isoniazid plus pyrazinamide (RHZ)-based regimen cures mice in 6 months, followed by relapse rates of 0-10%. On the basis of its recapitulation of outcomes in humans, the murine TB treatment model is the reference standard against which new treatments are compared.

Preclinical studies of tuberculosis treatment regimens containing rifapentine
Pre-clinical studies suggest that improved antimycobacterial activity can be achieved with rifamycin exposure greater than that of the current standard regimen in which rifampin is used at a dose of 10 mg/kg/dose (almost always given as 600 mg) once daily. Increased rifamycin exposure can be achieved by using rifapentine. Of note, the pharmacokinetics of rifapentine have been shown to be similar in mice and in humans (Rosenthal et al., 2005). In the murine model of TB treatment, once-daily rifapentine administered during intensive phase has very potent antimycobacterial activity that results in durable cure after only 3 to 4 months of total treatment (Rosenthal et al., 2008). After aerosol infection, mice achieved a bacillary burden of 7.21 $\log_{10}$ cfu per lung. Treatment with a standard regimen of daily rifampin (10 mg/kg) plus isoniazid and pyrazinamide resulted in a decrease in bacillary burden of approximately 3 logs at completion of 4 weeks of treatment. However, treatment with a regimen of daily rifapentine (10 mg/kg) plus isoniazid and pyrazinamide was significantly more active at 4 weeks (mean lung cfu counts that were 1.00 log10 cfu lower than those for the standard regimen, $p<0.001$) and at 8 weeks (mean lung cfu counts that were >2 log10 cfu lower than those for the standard regimen, $p<0.001$). Furthermore, after 12 weeks of treatment, 100% (15/15) of mice treated with the standard regimen had bacteriological relapse, compared to 0/15 mice treated with the rifapentine regimen. In fact, the rifapentine regimen resulted in cure of 15/15 (100%) of mice after treatment for only 10 weeks.

Thus, murine studies indicate that rifapentine administered daily during combination intensive phase treatment has potent antimycobacterial activity that is associated with ability to achieve durable cure without relapse after about 3 months of total treatment.

Clinical trials of daily rifapentine

Phase I
Dooley and colleagues conducted a phase I dose-escalation study among healthy adult volunteers to determine the safety and pharmacokinetics of escalating daily doses of rifapentine (Dooley et al, 2012). Participants received 5, 10, 15, or 20 mg/kg/dose rifapentine given once daily for 15 consecutive days; 20 mg/kg/dose was the pre-specified maximum dose; a cohort of additional participants received rifampin 10 mg/kg/dose. Of note, this study used strict weight-based dosing, such that the average rifapentine dose administered in the 20 mg/kg/dose cohort was 1650 mg daily. A total of 33 participants received study drugs. There were no grade 2 or higher clinical adverse events. Dose-limiting toxicities were observed in three participants, one each in the rifampin (grade 3 neutropenia), rifapentine 10 mg/kg (grade 3 serum liver transaminase elevation), and rifapentine 15 mg/kg (grade 3 lymphopenia) cohorts. In this study, the safety profile of rifapentine was similar to that of rifampin 10 mg/kg/dose, and it was concluded that rifapentine administered daily was tolerated and safe at doses as high as 20 mg/kg/dose. From a PK perspective, increases in rifapentine dosage resulted in less-than-dose proportional increases in single and multiple dose maximal concentrations.

Phase 2

The TBTC recently completed two phase 2 studies to assess the antimicrobial activity, safety, and tolerability of daily rifapentine administered with isoniazid, PZA, and ethambutol during the first eight weeks of pulmonary TB treatment. In TBTC Study 29, 531 adults with sputum smear positive pulmonary TB were randomized to receive rifapentine approximately 10 mg/kg/dose or rifampin 10 mg/kg/dose administered 5 days per week for 8 weeks (intensive phase) with isoniazid, PZA, and ethambutol; study drugs were administered on an empty stomach (Dorman et al, 2012). The co-primary endpoints were negative sputum cultures on liquid and on solid media at the end of intensive phase; safety and tolerability were also assessed. This study demonstrated no significant difference between regimens in antimicrobial activity based on the surrogate marker of culture status at completion of intensive phase (culture conversion on solid media 83.3% vs. 86.4% for the rifampin vs. rifapentine groups; and conversion in liquid media 65.1% vs. 67.9% in the rifampin vs. rifapentine groups). The rifapentine regimen was well tolerated based on similar proportions of participants discontinuing assigned treatment overall (15.7% in the rifampin group vs. 14.5% in the rifapentine group) or due to toxicity (1.2% in the rifampin group vs. 1.5% in the rifapentine group). There were no differences, by treatment group, in proportions of participants with a serious adverse event related to study treatment (0.4% in the rifampin group vs. 1.1% in the rifapentine group), or by type or severity of adverse events. Hepatitis occurred in 2.8% of rifampin group participants vs. 4.0% of rifapentine group participants (p=0.48). The investigators concluded that the rifapentine regimen administered on an empty stomach 5 days/week for 8 weeks was safe and well-tolerated but not significantly more active than the conventional rifampin regimen.

The TBTC subsequently conducted a randomized, multicenter, dose-ranging study to determine the optimal dose of daily rifapentine during the first 8 weeks of pulmonary TB treatment. In TBTC Study 29X, 334 adults with sputum smear positive pulmonary TB were randomized to receive rifampin (approximately 10 mg/kg/dose) or rifapentine (approximately 10, 15, or 20 mg/kg/dose, maximum dose 1500 mg) administered with a high fat meal once daily for 8 weeks, in addition to isoniazid, PZA, and ethambutol. Rifapentine was well-tolerated across all treatment arms based on a pre-specified definition and also based on comparison with the rifampin group. Percentages of participants discontinuing assigned treatment were: rifampin 11/85 (12.9%; upper bound of 90% one-sided CI 19.0); rifapentine 10 mg/kg 5/87 (5.7%; 10.5); rifapentine 15 mg/kg 5/81 (6.2%; 11.3); and rifapentine 20 mg/kg 9/81 (11.1%; 17.1). There were two deaths – one in the rifapentine 15 mg/kg group due to hematemesis, and one sudden death in the rifapentine 20 mg/kg group in a 61 year old male with untreated hypertension and diabetes mellitus and a strong family history of cardiac disease and sudden death. There were no differences between treatment groups in the percentages of participants with a serious adverse event associated with study treatment, or by type or severity of adverse events. Serious adverse events attributed to study treatment were as follows, by treatment assignment: two events among 85 participants in the rifampin group (one hepatitis, one drug allergy); one event among 87 in the rifapentine 10 mg/kg group (leukocytosis); no events among 81 participants in the rifapentine 15 mg/kg group; one event among 81 participants in the rifapentine 20 mg/kg group (hepatitis). With respect to antimicrobial activity (efficacy), the rifapentine regimens were substantially more active than the standard rifampin regimen based on week 8 (end of intensive phase) culture status (Table 1) as well as time to stable culture conversion.

Antimicrobial activity was associated with rifapentine exposure (area under the concentration time curve [AUC]) (Table 1B). The higher rifapentine exposures were associated with very high rates of sputum sterilization at two months, a very good indicator of overall efficacy of an anti-tuberculosis regimen. Findings were consistent with a steep exposure-response relationship. Pharmacodynamic models were used to further elucidate the relationships between antimycobacterial activity and assigned rifapentine treatment arm, administered rifapentine dose, and rifapentine drug exposure. For efficacy outcomes of

time to stable culture conversion in solid media and time to stable culture conversion in liquid media, there was a significant association with rifapentine AUC (p=0.0002 for solid media and p=0.001 for liquid media) but not for assigned rifapentine mg/kg group (p=0.6 for solid media and p=0.36 for liquid media) or for administered rifapentine dose in mg (p=0.17 for solid media and p=0.17 for liquid media). For rifapentine, the exposure-response relationship was best described by a sigmoidal Emax function (Figure 1), with the greatest change in effect per change in exposure occurring between exposures of approximately 200 mcg*h/L and 550 mcg*h/L, with a plateau in efficacy at higher exposures. In addition to identifying a target AUC of approximately 500 to 600 mcg*h/L, pharmacokinetic studies yielded additional information pertinent for rifapentine dosing. Specifically, the relationship between participant body weight and rifapentine clearance was examined, and clearance was not significantly affected by body weight, thereby supporting 'flat' dosing of rifapentine (i.e. rifapentine dose is not adjusted for body weight) (Figure 2). In addition, pharmacokinetic/pharmacodynamic modeling also predicted that a rifapentine dose of 1200 mg without food would yield an AUC of approximately the same as that of a rifapentine dose of 900 mg with a very high fat meal. Given that target rifapentine AUC lies somewhere between that achieved with a very high fat meal and rifapentine dose of 900 to 1200 mg, the strategy proposed in the current phase 3 trial is a rifapentine dose of 1200 mg with a modest food requirement, with the rationale that a very high fat meal is poorly feasible under phase 3 trial or routine TB care conditions whereas a more general recommendation of dosing with food is likely to be broadly feasible.

Table 1A. Percentages of participants with negative cultures at completion of intensive phase treatment, by treatment assignment, for the modified intention-to-treat analysis group, S29X

	Rifampin	**Rifapentine 10 mg/kg**	**Rifapentine 15 mg/kg**	**Rifapentine 20 mg/kg**
Solid culture medium				
% (n/n) with negative cultures	81.3 (52/64)	92.5 (62/67)	89.4 (59/66)	94.7 (54/57)
% difference vs. Rifampin		11.3	8.1	13.5
(95% CI)		(-1.7, 24.3)	(-5.5, 21.8)	(0.6, 26.3)
p-value		0.10	0.29	0.05
Liquid culture medium				
% (n/n) with negative cultures	56.3 (36/64)	74.6 (50/67)	69.7 (46/66)	82.5 (47/57)
% difference vs. Rifampin		18.4	13.4	26.2
(95% CI)		(0.8, 35.9)	(-4.5, 31.4)	(8.9, 43.5)
p-value		0.04	0.16	<0.01

Table 1B. Percentages of participants with negative cultures at completion of intensive phase treatment, by rifapentine area under the concentration-time curve tertile, for the modified intention-to-treat analysis group, S29X

	Rifampin	**Rifapentine AUC ≤ 323 mcg*h/L**	**Rifapentine AUC 324 to 513 mcg*h/L**	**Rifapentine AUC > 513 mcg*h/L**
Solid culture medium				
% (n/n) with negative cultures	81.3 (52/64)	83.9 (52/62)	100.0 (63/63)	92.3 (60/65)
% difference vs. Rifampin		2.6	18.8	11.1
(95% CI)		(-12.2, 17.4)	(7.6, 29.9)	(-2.0, 24.2)
p-value		0.88	<0.01	0.11
Liquid culture medium				
% (n/n) with negative cultures	56.3 (36/64)	54.8 (34/62)	90.5 (57/63)	80.0 (52/65)
% difference vs. Rifampin		-1.4	34.2	23.8
(95% CI)		(-20.4, 17.5)	(18.5, 50.0)	(6.6, 40.9)
p-value		1.00	<0.01	<0.01

Figure 1. Exposure-response relationship for rifapentine

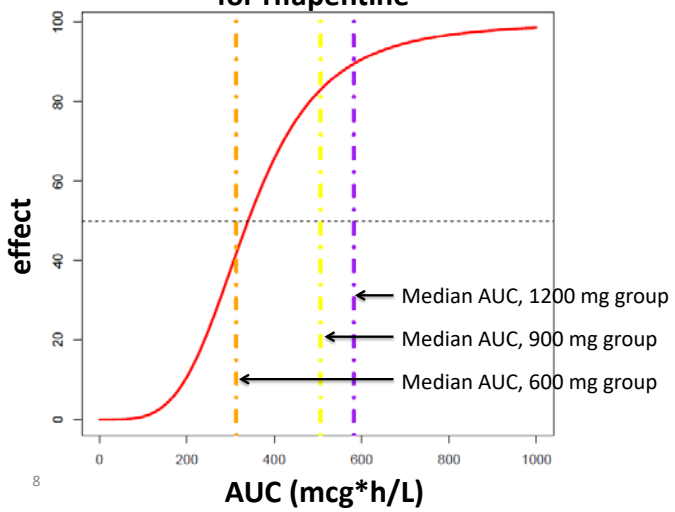
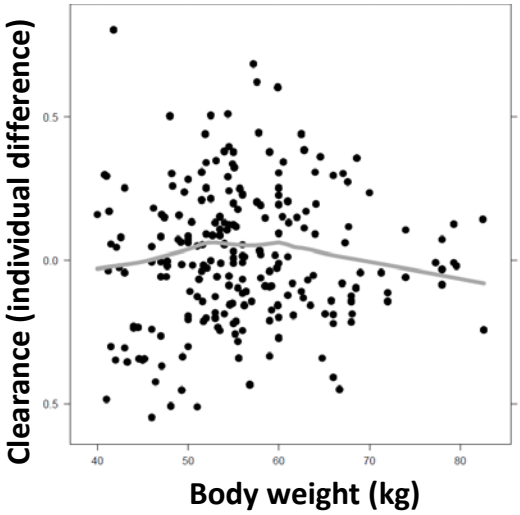


Figure 2. Rifapentine clearance is not meaningfully affected by body weight



Moxifloxacin for TB treatment

Moxifloxacin is a fluoroquinolone with potent activity against *M. tuberculosis* in vitro and in animal models, including sterilizing activity in animal models. In animal models, moxifloxacin's potent activity is partly explained by the fact that it accumulates in granulomas and pulmonary lesions at higher concentrations than found in plasma and lung tissue (Kjellson et al., 2012; Prideaux et al., 2011). In humans, data on the long-term use of moxifloxacin have shown that it has an excellent safety profile. Three phase 2 TB clinical trials have shown that substitution of moxifloxacin for ethambutol during the intensive phase of pulmonary TB treatment increases the antimicrobial activity of the regimen, as assessed using surrogate markers (Burman et al., 2006; Rustomjee et al., 2008; Conde et al., 2009). A recently completed phase 3 trial found that treatment with a weekly regimen of rifapentine and moxifloxacin during the continuation phase of therapy (Rifaquin 6 month regimen, Jindani, 2014) was non-inferior to daily isoniazid plus rifampin; the efficacy of rifapentine plus moxifloxacin in this trial is significant because once-weekly rifapentine with isoniazid (instead of moxifloxacin) is associated with higher rates of relapse and treatment failure (Vernon et al., 1999). Two phase 3 treatment shortening studies using fluoroquinolone-based 4-month regimens administered daily have been completed recently. The Oflotub Trial was an open-label, Phase 3 multicenter trial evaluating the efficacy and safety of a 4-month gatifloxacin (G) containing regimen compared to the standard 6-month HRZE regimen (Merle et al, 2014). The Oflotub 4 month regimen consisted of a 2 month intensive phase of GHRZ, followed by a 2 month continuation phase of GRH (2GRHZ/2GRH) versus a control arm of 2ERHZ/4RH, administered 6 days per week. The investigational 4 month regimen failed to achieve non-inferiority at a 6% margin: in a modified intention-to-treat analysis unfavorable outcomes at 24 months from end-of-treatment had occurred in 21.0% (146/694) in the gatifloxacin-containing arm vs. 17.2% (114/662) in the control arm (difference +3.5%, 95% CI -0.7% to +7.7%). The REMox phase 3 study was a randomized placebo-controlled double-blind trial comparing two treatment shortening regimens, namely 2MHRZ/2MHR and 2EMRZ/2MR, with the standard control regimen 2EHRZ/4HR (Gillespie et al 2014). The primary end point was treatment failure or relapse within 18 months after randomization. In the REMox study, neither of the investigational

moxifloxacin-containing regimens was shown to be non-inferior to the control. Specifically, of the 1931 patients who underwent randomization, in the per-protocol analysis, a favorable outcome was reported in fewer patients in the 2MHRZ/2MHR group (85%) and the 2EHRZ/4HR group (80%) than in the control group (92%), for a difference favoring the control group of 6.1 percentage points (97.5% confidence interval [CI], 1.7 to 10.5) versus the 2MHRZ/2MHR group and 11.4 percentage points (97.5% CI, 6.7 to 16.1) versus the 2EHRZ/4HR group. Overall the 2MHRZ/2MHR group performed slightly better than the 2EHRZ/4HR group, and achieved non-inferiority in certain sub-groups (e.g. females). In the REMox study the two moxifloxacin-containing regimens produced a more rapid initial decline in bacterial load as compared to the control group. As in the Oflotub Trial, however, overall the experimental moxifloxacin single-substitution regimen was also not shown to be non-inferior to the control. The fluoroquinolone-containing regimens were safe and well-tolerated in both the Oflotub (gatifloxacin) and the REMox (moxifloxacin) studies. Overall, pre-clinical and clinical studies have shown that the single substitution of moxifloxacin for ethambutol increases antimicrobial activity of the regimen, but this increase is not sufficient to achieve acceptable cure rates after truncation of therapy to four months (Burman et al., 2006; Rustomjee et al., 2008; Conde et al., 2009, Merle et al, 2014, Gillespie et al., 2014).

Quantitative capability of the Xpert MTB/RIF assay and its correlation with smear microscopy and culture.

The Xpert MTB/RIF Assay, endorsed by the WHO in 2010 and FDA approved for marketing in the US in 2013 for diagnosing TB, simultaneously detects the presence of *M. tuberculosis* in sputum and determines if genetic markers for rifampin resistance are present. Two studies have compared Xpert MTB/RIF results with sputum smear results in newly suspected pulmonary TB (Blakemore et al, 2011; Friedrich et al 2011). Medium and high qualitative readings from Xpert MTB/RIF correlate well with finding acid fast bacilli on sputum smears. The quantitative capability of the Xpert MTB/RIF assay will be used in this trial at baseline to permit participant randomization based on a medium or high semi-quantification of *M. tuberculosis* copies on their Xpert test at screening. Drug susceptibility results as provided by the Xpert MTB/RIF assay or the Hain MTBDR*plus* assay, will also be used as part of screening and enrollment procedures.

Efavirenz and Rifapentine Drug-Drug Interactions

Rifamycin antibiotics such as rifapentine have the potential to cause significant drug-drug interactions with antiretroviral therapy. Rifapentine is a known inducer of various cytochrome P450 iso-enzymes. The nonnucleoside reverse transciptase inhibitor efavirenz is a cytochrome P450 substrate, leading to concern for decreased efavirenz concentrations and an increased risk of virologic failure if dosed concurrently with rifapentine. Exposure-response relationships between efavirenz concentrations and virologic failure have been demonstrated. For example, Cohen et al. reported that in an evaluation of 142 HIV-infected persons, efavirenz mid-dosing interval concentrations <1 mg/L were strongly associated with an increased risk for virologic failure (odds ratio 12.5, 95% CI, 2.7-57.3) (Cohen, 2009). The collective data for efavirenz indicate an increased risk of virologic failure if mid-dosing interval (or trough) concentrations are less than 1 mg/L.

Clinical studies evaluating the effects of rifapentine co-administration on efavirenz pharmacokinetics and efficacy have shown mixed results. Among individuals with HIV infection enrolled in a clinical trial of treatment for latent TB infection, preliminary data recently presented by Podany et al. suggest no clinically relevant increase in efavirenz oral clearance when dosed together with isoniazid and rifapentine (10 mg/kg) once daily for four weeks. In this study of 87 patients, median mid-dosing interval efavirenz concentrations decreased in the presence of rifapentine (2588ng/mL vs 2460ng/mL), suggestive of an

induction effect on efavirenz by rifapentine; however, the geometric mean ratio of efavirenz oral clearance increased by only 4%. Additionally, virologic suppression was maintained in 97% of the patients in the study (Podany, 2014). A second study, from Farenc et al. investigated the effect of repeated once weekly 900mg rifapentine dosing on efavirenz pharmacokinetics. In 12 HIV-infected, TB free adults, the authors found a minimal decrease in efavirenz exposure, as measured by a mean decrease in AUC_{0-24} of 14% (Farenc, 2014). A third study investigated the effect of daily rifapentine dosing (15mg/kg) for 21 days in HIV-infected, TB free adults receiving efavirenz based ART with baseline suppressed viral load (VL< 20 copies/mL). After a single dose of rifapentine efavirenz PK was unchanged (Cmax, AUC_{0-24}, Cmin). However, after 21 once-daily doses of 15 mg/kg rifapentine, decreases of 17%, 37% and 33% were seen in Cmax, AUC_{0-24} and Cmin respectively (Sanofi, 2014). All patients maintained viral suppression while taking RPT. While these studies are encouraging in that co-administration of rifapentine at daily doses of 10 and 15 mg/kg with EFV-based ART did not appear to increase risk of HIV treatment failure, further investigations of efavirenz PK and HIV treatment response when rifapentine is given at 1200 mg daily for a longer duration are needed.

The Tuberculosis Trials Consortium (TBTC)
The mission of the TBTC, funded by the U.S. Centers for Disease Control and Prevention, is to conduct programmatically relevant clinical, laboratory and epidemiologic research concerning the diagnosis, clinical management, treatment and prevention of tuberculosis infection and disease. The TBTC has sites in the United States, Spain, South Africa, Hong Kong, Kenya, Vietnam, Peru, and Uganda. All sites have close connections with the local TB control program; some sites are based in the TB control program. All sites work with experienced mycobacterial laboratories, and the CDC's Mycobacteriology Laboratory serves as the central laboratory for confirmation of drug-susceptibility testing, DNA fingerprinting, and further characterization of drug-resistant isolates. Since its inception in 1994 over 13,000 patients have been enrolled in TBTC clinical trials.

The AIDS Clinical Trials Group (ACTG)
The mission of the ACTG, established in 1987, is to develop and conduct scientifically rigorous translational research and therapeutic clinical trials, in the U.S. and internationally, related to HIV infection and its complications including tuberculosis. The ACTG is funded by the U.S. National Institutes of Health through the National Institute of Allergy and Infectious Diseases. ACTG units and investigators serve their communities as major resources for HIV/AIDS research, treatment, care, and education. The ACTG tuberculosis laboratory infrastructure consists of one international tuberculosis specialty laboratory as well as regional tuberculosis diagnostic laboratories.

2.2 Rationale

Rationale for regimen selection
The current standard six-month TB treatment regimen for drug-susceptible pulmonary tuberculosis is associated with unacceptably high rates of treatment default under program conditions, thereby contributing to individual morbidity and mortality, *M. tuberculosis* transmission, and drug resistance. Highly potent regimens of shorter treatment duration may facilitate treatment completion and direct observation of treatment, thereby improving individual and public health. Studies using animal models of TB chemotherapy have shown a clear relationship between rifamycin exposure and reduction of bacillary burden. Animal studies also indicate that rifapentine-based regimens are highly potent and can reduce overall tuberculosis treatment duration to approximately 3 months. In humans, phase 1 and 2 clinical trials support the safety and tolerability of rifapentine at daily doses up to 20 mg/kg. A phase 2 clinical trial has shown a strong drug exposure-response effect for rifapentine using a surrogate marker of time to

stable culture conversion. These results provide rationale for a phase 3 clinical trial to determine the efficacy, using the definitive endpoint of durable cure, of a regimen containing rifapentine substituted for rifampin and administered in combination with other drugs for 17 weeks (approximately four months).

This trial will also assess the efficacy of a second investigational 17-week regimen that incorporates two strategies to enhance antimicrobial activity. The first strategy is optimization of rifamycin exposure through the single substitution of high dose rifapentine for rifampin throughout treatment as described above. The second strategy is a dual substitution approach that seeks to further enhance the potency of the regimen by also replacing ethambutol (which has relatively weak activity) with moxifloxacin, in the context of optimized rifamycin exposure. As described above, available evidence from animal models and in humans indicates that moxifloxacin, when substituted for ethambutol, will contribute to regimen bactericidal activity, even though that single substitution alone is insufficient to shorten treatment to four months. The investigational regimen that contains both rifapentine and moxifloxacin may well be the most potent regimen possible without new chemical entities. Therefore if both investigational regimens fail in the proposed study, then the implications are that new drugs are required for treatment shortening, and that treatment shortening cannot be achieved with existing drugs. Such a finding would push the drug-susceptible TB therapeutics field in a different direction.

Rationale for dosing strategy
With respect to rifapentine dose and dosing strategy, this trial will use a flat dose of 1200 mg with food dosed daily. This is based on 1) demonstration of safety of rifapentine at 1200mg in phase 1 and 2 trials, 2) demonstration that body weight does not significantly affect rifapentine clearance, 3) recognition of an effect of food in substantially increasing rifapentine absorption (Zvada et al., 2010) and 4) modeling predictions that the target rifapentine exposure (AUC of approximately 500 to 600 mcg*h/L) is achievable using this strategy.

For rifampin, administration with food slows the rate of absorption and decreases the maximal concentration (Cmax) by about 36% in healthy adults but to a lesser extent (5 to 15%) in TB patients, with little to no effect on AUC (reduction of 6% in healthy adults; reduction of 4% to increase of 8% in TB patients) (Peloquin et al., 1999; Zent et al., 1995). Overall the clinical consequences of these PK effects are unclear. As a consequence rifampin will not be dosed with food.

Rationale for an open-label (not blinded) study
This study will be open-label; participants, study staff, and clinical care providers will have knowledge of treatment assignment. While blinding is often incorporated into clinical trials as a strategy to reduce bias, blinding is not without cost. Besides logistic complexity, there are two main reasons to not incorporate blinding into this trial. First, if blinding through use of placebos were to be incorporated into this trial, the already substantial pill-burden would be further increased to approximately 20 pills per day, which will be difficult for participants to tolerate. Poor tolerability of the pill burden may result in diminished adherence to treatment and in turn efficacy. Besides tolerability, from a biological perspective the dissolution of pills in the gastrointestinal tract may be reduced with such a high pill burden, a situation that could result in diminished treatment efficacy and increased risk for acquisition of drug resistance. Reduced bioavailability due to the burden of added placebo pills also might reduce the generalizability of study findings to possible subsequent programmatic usage. As noted above, there may be a differential effect of food on rifampin and rifapentine such that different food advice is required so as to optimize regimen pharmacokinetics and potential efficacy. The study will minimize any impact of ascertainment bias through the use of objective laboratory measures for the primary efficacy endpoint as well as incorporation of a central study clinician (who is blinded as to individual participants' treatment

assignments) to advise local site teams in instances of potential toxicity or treatment failure/recurrence. Co-intervention bias is unlikely during the active treatment in the first four months, and it is unlikely that the use of placebo can effectively avoid contamination bias in the last two months. Co-intervention bias is unlikely during active TB treatment, as site teams will closely monitor participants. Measures will be taken to evaluate participants equally, regardless of assigned study arm, such that timing of study visits and endpoints will be applied uniformly.

Rationale for CD4 testing before enrollment

HIV-infected individuals will be excluded from enrollment if, at the time of enrollment, their CD4 T cell count is known to be <100 cells/mm$^{3.}$ The rationale for doing so is the challenge of starting concomitant treatment for HIV-infection and TB within a short period of time as recommended by WHO guidelines, the potential for severe immune reconstitution when antiretroviral therapy is initiated early in the course of anti-TB therapy, an increased probability for the diagnosis of conditions that would require therapy with medications that have drug-drug interactions with study drugs, and the indication for primary antimicrobial prophylaxis against opportunistic infections (such as toxoplasmosis and *Mycobacterium avium*) with consequent increase in the probability of drug-drug interactions and adverse events. Additionally, treatment assignment is stratified by CD4 T cell count (Section 7.2).

Rationale for including adolescents

Inclusion of children in clinical trials of tuberculosis treatment increasingly has been called for to support the rational use and increased availability of anti-tuberculosis medications for children (Burman 2006, McKenna 2014). Tuberculosis disease characteristics, presentation, diagnosis, and treatment are similar for adults and adolescents.

Rifapentine currently is approved in the United States for use in persons as young as 12 years old (Priftin® package insert). Rifapentine pharmacokinetic results have been found to be similar between adults and adolescents down to this age (Marshall 1999). Rifapentine 900 mg once weekly, with isoniazid for 12 weeks has been used to treat children for latent TB infection (LTBI) (Villarino 2015): the rifapentine-containing regimen was found to be as safe and effective as a 9-month daily isoniazid regimen, among 552 children age 2-17 years old treated with the rifapentine-containing regimen. Based on a large clinical trial that reported treatment of patients as young as 12 years old (Sterling 2011), CDC recommends use of this rifapentine-containing regimen “as an equal alternative to 9 months of daily self-supervised INH for treating LTBI in otherwise healthy patients aged ≥12 years.” (CDC 2011) Although the phase 2 trial of daily high-dose rifapentine enrolled 81 participants ≥ 18 years old (Dorman 2013), available animal and human data and a formal review by Sanofi support the safety of including adolescents in this clinical trial (Marilyn Maroni personal communication).

Moxifloxacin usage in children has been limited because of findings that treating juvenile dogs with doses higher than recommended for humans (≥ 30 mg/kg/day) resulted in arthropathy (Avelox® package insert). Moxifloxacin has been recommended and used in children for treatment of multidrug resistant (MDR) TB although few safety data have been collected systematically (Bradley 2011). One recent report of a series of 9 children age 6 months to 13 years, treated with moxifloxacin for a range of 3-16 months, attributed side effects possibly to moxifloxacin in 2 patients (Garazzino 2014): A 6-year old girl developed arthritis of the ankle after 3 months, which “spontaneously resolved few days after drug cessation.” A 3-year old girl developed “grade three elevation of liver function tests after 9 months of treatment.” A more recent report of 23 children, median age 11.1 years (IQR 9.2-12.0 years), treated for MDR TB with moxifloxacin followed the children for a median of 236 days (IQR 142-541 days (Thee 2014). This group found lower exposures in children than in adults following an oral dose of 10 mg/kg and lower exposure with HIV infection. They found moxifloxacin to be safe and well tolerated, with a conservative approach to determining an event not to be related to moxifloxacin in the presence of multiple second line drugs. Adverse events possibly, probably, or definitely related to moxifloxacin were arthralgia (4 patients, 5

events, 4 grade 1, 1 grade 3), pain other than traumatic injury (1, grade 1), headache (5 patients, 5 events, 4 grade 1, 3 grade 3), fatigue/malaise (1, grade 1), nausea (9 patients, 9 events) grade 1), vomiting (3 patients, 3 events, 3 grade 1),cutaneous reaction (1, grade 1), pruritis,(2 patients, 4 events, 4 grade 1), elevated alanine-aminotransferase (ALT) (4 patients, 5 events, 2 grade 1, 2 grade 2 1 grade 3), bilirubin (1 grade 2). ECG data was available for 13 children: mean QTc was 403 ms (SD 30 ms); none had QTc interval >450ms. In a separate report, a case of self-limited polyarthritis was reported for a 12-year old child who inadvertently received a single 2 gram dose of moxifloxacin (50 mg/kg/day) (Torres 2008), illustrating the presence of some risk but also the difficulty of finding moxifloxacin toxicity in children with standard dose treatment.

Daily treatment of adolescents and children with moxifloxacin and with high-dose rifapentine has been limited. The benefits of finding regimens that would substantially shorten treatment for this important subset of TB patients outweigh the theoretical risks. The frequent careful monitoring for signs and symptoms in a clinical trial provides the opportunity to respond quickly to abnormal findings if they occur in an individual, but also to document systematically the safety profile for these promising treatment regimens.

2.3 Potential Harms and Benefits

2.3.1 Potential harms

Risks associated with study participation include the possibilities that an investigational regimen has efficacy that is inferior to the standard regimen and/or an investigational regimen is more toxic than the standard regimen, and confidentiality risks.

The investigational regimens could prove to have inferior efficacy compared to the standard regimen. TBTC Study 29X showed that a regimen containing rifapentine administered daily at 1200 mg (approximately 20 mg/kg) has sufficient antimicrobial activity to justify further evaluation compared to a regimen containing rifampin administered daily at approximately 10 mg/kg, using a surrogate marker for durable cure. Other recent phase 2 studies have demonstrated that substitution of moxifloxacin for ethambutol increases the antimicrobial activity of the regimen. Equipoise around whether these increases in antimicrobial activity can result in durable cure after four months of treatment forms the basis for inclusion of test regimens 2PHZE/2PH and 2PHZM/2PHM. Further, as described above, studies have shown that moxifloxacin substituted for ethambutol increases antimicrobial activity, and PZA has important bacterial sterilizing properties. The following measures will minimize risk to participants: individual participants will be closely monitored clinically and bacteriologically, an interim efficacy analysis will be performed, and a Data and Safety Monitoring Board will review trial progress regularly. In addition, it is anticipated that participants who are not cured will be re-treatable with conventional anti-tuberculosis drugs as acquired drug resistance is very unlikely in the setting of highly monitored directly observed combination therapy (Johnson et al., 2009, Jindani et al., 2013).

The investigational regimens could prove to have greater toxicity than the standard regimen. This is unlikely given that toxicity data collected in the phase 2 clinical trial showed that rifapentine administered at a once daily dose of 20 mg/kg for eight weeks was well tolerated. However, no clinical trial has included patients treated with daily rifapentine for more than 2 months. With respect to the moxifloxacin-containing regimen, the toxicity profile of moxifloxacin is well known. Moxifloxacin is used regularly for prolonged periods during the treatment of patients with drug resistant tuberculosis. Participants in this clinical trial will be monitored closely for adverse events. The Data and Safety Monitoring Board (DSMB) will review safety data regularly.

Confidentiality risks will be minimized through the measures described in Section 15.3.

2.3.2 Potential benefits

All 3 study treatment regimens are offered with therapeutic warrant, meaning that it is reasonable to expect that these TB treatments will cure TB at an acceptable, if not yet uniformly demonstrated, rate. Study participants will benefit indirectly as it is well established that tuberculosis outcomes for participants in tuberculosis treatment trials are better than those for patients receiving routine care. This study will benefit society by contributing to the understanding of optimal strategies for treating tuberculosis. The inclusion of clinical specimen storage along with the combined use of two culture media types (solid and automated liquid) in this phase 3 trial will contribute urgently needed data that can help determine the optimal use of liquid culture in drug development and will facilitate discovery efforts for new surrogate markers.

3 DESCRIPTIONS OF STUDY DRUGS

Drugs to be administered during this study include the following: rifapentine, rifampin, moxifloxacin, isoniazid, pyrazinamide, ethambutol, and vitamin B6 (pyridoxine). Rifapentine and the other drugs are described in detail in their package inserts. Key points are summarized below.

3.1 Rifapentine

Rifapentine is indicated for the treatment of pulmonary tuberculosis caused by *Mycobacterium tuberculosis*, and must be used in combination with one or more antituberculosis drugs to which the bacterial isolate is susceptible (Priftin package insert, 2010). Rifapentine, as Priftin® was approved by the U.S. FDA in 1998. FDA approval was based on the results of "Clinical Study 008", an open-label, prospective, randomized study of 722 patients with active pulmonary TB (Priftin package insert, 2010). Rifapentine is a semisynthetic rifamycin derivative with a microbiologic profile similar to that of rifampin. Its structure differs from that of rifampin by the presence of a cyclopentyl ring instead of a methyl group at the piperazinyl moiety. It has a longer half-life than rifampin, and, like rifampin, rifapentine inhibits bacterial RNA synthesis by binding to the β-subunit of DNA-dependent RNA polymerase. Rifapentine is well absorbed from the gastrointestinal tract, with 70% bioavailability; when taken with food, its AUC and C_{max} increase by 43% and 44%, respectively (Priftin package insert, 2010). It reaches peak concentrations in the serum 5 to 6 hours after ingestion. Rifapentine and its 25-desacetyl metabolite are highly protein-bound, 97.7% and 93%, respectively, primarily to albumin. Rifapentine is metabolized by an esterase enzyme found in the liver and blood to 25-desacetylrifapentine, a microbiologically active metabolite that contributes about 40% of the drug's overall activity. For *M. tuberculosis*, the MIC of 25-desacetyl rifapentine is 0.25 mcg/mL, while that of rifapentine is 0.05 mcg/ml. The drug and the active metabolite have half-lives of 14-17 and 13 hours, respectively. The drug is excreted in bile and eliminated in feces. Less than 10% of rifapentine is excreted in the urine as unchanged drug. Rifapentine, like other rifamycins, induces CYP3A4, 2C8, and 2C9, which can lead to more rapid metabolism and clearance of many drugs. Rifamycins are also known to induce the activity of phase II enzymes such as glucuronosyltransferase and sulphotransferase and may reduce levels of drugs metabolized by those pathways. Rifapentine is available as 150 mg tablets. Rifapentine, like other rifamycins, causes red-orange discoloration of body fluids and can stain contact lenses. In clinical trials in which rifapentine was combined with isoniazid and other antituberculosis drugs and administered once or twice weekly, rates of adverse reactions were similar with rifampin and rifapentine, with increased liver aminotransferase activity in about 5% of patients. The only adverse effect that has occurred more often with rifapentine than with rifampin has been hyperuricemia when the drug was given twice-weekly; of note, hyperuricemia was attributed to pyrazinamide that was administered concomitantly. Other adverse reactions that occurred in 1-5% of patients included the following: hemoptysis, dizziness, hypertension, headache, gastrointestinal upset, rash, cytopenias, hematuria, pyuria, and proteinuria (Priftin package insert, 2010).

3.2 Rifampin

Rifampin is a semi-synthetic rifamycin derivative that is highly active against mycobacteria, most gram-positive bacteria, and some gram-negative bacteria. It is bactericidal for both intracellular and extracellular microorganisms. By inhibiting prokaryotic DNA-dependent RNA polymerase, it suppresses the early elongation of the nucleotide chain in RNA synthesis. Rifampin 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 8-20 mcg/ml. The half-life of rifampin varies from 2 to 5 hours, and it is shortened by approximately 20-40% after the first week of daily treatment because of the induction of hepatic microsomal enzymes. The half-life is unaffected by renal impairment but is increased by liver disease or biliary obstruction. Rifampin is deacetylated to an enterohepatically-recirculated active metabolite, and 50% to 60% is excreted in the feces. Up to 30% of a dose is excreted in the urine. Approximately 85% of circulating rifampin is bound to plasma proteins, and is widely distributed throughout the body. Rifampin is a potent inducer of a number of hepatic enzymes involved in the metabolism of drugs and some hormones (Venkatesan, 1992). This enzyme induction causes more rapid elimination (and potential loss of efficacy) of many drugs. In the usual daily doses of 10 mg/kg (maximum 600 mg), rifampin is well tolerated. It often causes harmless but disconcerting red-orange discoloration of tears, sweat, saliva, feces, and urine. Less than 4% of TB patients experience significant adverse reactions to rifampin. Gastrointestinal adverse effects are the most common, and they include epigastric distress, anorexia, nausea, vomiting, cramps, and diarrhea. Hepatitis rarely occurs in persons who have normal baseline hepatic function. The incidence of hepatitis may be increased for older persons and those who have chronic liver disease or alcoholism, but remains substantially lower than that for pyrazinamide or isoniazid. Rifampin can cause a flu-like syndrome of fever, chills, headache, dizziness, and bone pain, although this is uncommon using the 600 mg dose given daily. In a very small proportion of patients the flu-like syndrome is associated with interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. (Rifadin [rifampin] package insert. Sanofi, 2013).

3.3 Moxifloxacin

Moxifloxacin (Avelox®, Bayer HealthCare Pharmaceuticals, Inc) is a fluoroquinolone antibacterial that is distinguished by a methoxy group at the C-8 position and an S,S-configured diazabicyclononyl ring moiety at the C-7 position. The mechanism of action against *M. tuberculosis* is by inhibition of the DNA gyrase enzyme involved in DNA replication. Moxifloxacin is well absorbed, with a bioavailability of approximately 90% (Avelox package insert, 2012). Pharmacokinetics are linear in the range of 50-800 mg single dose and up to 600 mg once daily dosing over 10 days. Steady state is reached within 3 days. The mean ($\pm$SD) maximum concentration (C_{max}) and AUC values at steady state with a 400 mg once-daily dosage regimen are 4.5$\pm$0.53 mcg/ml and 48$\pm$2.7 µg*h/ml, respectively. T_{max} is approximately 1-3 hours. The mean ($\pm$SD) plasma half-life is 12.1 $\pm$ 3.1 hours. Trough plasma concentration at steady state (400 mg once daily) is 0.95 $\pm$ 0.10 mcg/L. Co-administration with food may slightly prolong T_{max}, and may reduce the C_{max} by 16%; these effects are not believed to be clinically significant, and thus moxifloxacin can be administered with or without food. Moxifloxacin is 50% bound to plasma proteins. It is widely distributed, with some tissue concentrations reported in excess of plasma levels. Moxifloxacin is metabolized by glucuronide and sulfate conjugation. The cytochrome p450 enzyme system is not involved in the metabolism of moxifloxacin, nor does the drug effect it. Specifically, moxifloxacin does not affect CYP3A4, CYP2D6, CYP2C9, CYP2C19, or CYP1A2. Moxifloxacin should not be administered at the same time as antacids containing magnesium and/or aluminum, sucralfate, antidiarrheals that contain kaolin, or iron and/or zinc containing medications or supplements. As a class, fluoroquinolones are considered to be safe and relatively free of serious adverse effects (Ball, 1989). The safety and efficacy of moxifloxacin for the treatment of community acquired bacterial infections has been extensively studied. In the usual daily doses of 400 mg/day, moxifloxacin is well tolerated. Expected minor adverse events have occurred with a frequency similar to that for other fluoroquinolones in similar populations. Gastrointestinal adverse effects are the most common, and they include nausea (7%), diarrhea (6%), dizziness (3%), abdominal pain (2%), vomiting (2%), dyspepsia (1%), and taste perversion (1%). There are no current reports of hepatitis, although abnormal liver function tests have been noted in 1% of

patients. Of note, fluoroquinolones are generally used short-term for acute conditions. However, fluoroquinolones have been found to be safe and effective in long term use for chronic infections such as osteomyelitis, prostatitis, or chronic urinary tract infection, or skin and skin structure infections (Ball, 1989; Segev et al., 1999). In addition, Valerio et al. found that the combination of moxifloxacin, isoniazid, and rifampin given for 6 months was well tolerated (Valerio et al., 2003). For treatment of MDR-TB, a later-generation fluoroquinolone such as moxifloxacin is recommended for a duration of 8 months or more (WHO, 2011). Moxifloxacin causes a mild prolongation of the corrected QT (QT_c) interval. The mean effect on QT_c interval in 787 patients in Phase 3 clinical trials was 6 $\pm$ 26 milliseconds, but no morbidity or mortality was attributable to QT_c prolongation. In a review of over 6,000 patients treated with moxifloxacin, there were no reports of changes in the frequency of QT_c prolongation (Ball, 2000). In over 10 million patients treated with moxifloxacin there has been no evidence for an increased incidence of ventricular arrhythmia when compared to the overall population. Finally, the incidence of sudden death and syncope, both markers for sudden cardiac events, is not increased when compared to the incidence on the general population. For these reasons, electrocardiographic monitoring is no longer recommended for clinical trials involving moxifloxacin. In this study, moxifloxacin will not be used among patients with a known history of prolongation of the QT interval, patients with uncorrected hypokalemia and patients receiving class Ia (e.g. quinidine, procainamide) or class III (e.g. amiodarone, sotalol) antiarrhythmic agents, due to the lack of clinical experience with the drug in these patient populations (see Eligibility criteria). Pharmacokinetic studies between moxifloxacin and other drugs that prolong the QT interval such as cisapride, erythromycin, antipsychotics and tricyclic antidepressants have not been performed. An additive effect of moxifloxacin and these drugs cannot be excluded, therefore moxifloxacin should be used with caution when given concurrently with these drugs. The estimated incidence of fluoroquinolone-induced tendinopathy is 15 to 20 per 100,000. Fluoroquinolone-induced tendinopathy is diagnosed by a sudden onset of swelling and tenderness concurrent with or shortly after fluoroquinolone therapy, which is accompanied by tendon rupture in about 33% of all cases. The main site affected is the Achilles tendon, though tendinitis has been reported to involve the shoulder, knee, hand, and plantar aponeuroeses. Achilles tendon ruptures have been noted even months after discontinuation (Pierfitte et al., 1996). Concomitant use of corticosteroids is considered to be a risk factor for developing tendinopathy while taking fluoroquinolones. In over 10 million patients treated with moxifloxacin only 3 patients with tendon rupture have been reported. All had received concomitant corticosteroid treatment.

3.4 Isoniazid

Isoniazid is the hydrazide of isonicotinic acid and is one of the primary drugs for TB treatment. The activity of isoniazid is limited to the mycobacteria of the *M. tuberculosis* complex; it is bactericidal for rapidly dividing organisms and bacteriostatic for “resting” bacilli. The probable mechanism of action is the inhibition of the biosynthesis of mycolic acids, a component of the mycobacterial cell wall. Isoniazid is generally well absorbed; food and antacids decrease the rate, but not the extent of absorption. Peak blood levels of isoniazid, 3 to 5 mcg/ml, are obtained 30 minutes to 2 hours after ingestion of routine doses (Peloquin et al. 1999). It diffuses into all body fluids and cells and penetrates into the caseous material of a tuberculoma or pulmonary cavity. In the liver, it is acetylated to inactive metabolites, and 75% to 95% of the dose is excreted as inactive metabolites in the urine within 24 hours. Isoniazid clearance rates depend on 2 metabolic phenotypes, slow and fast acetylation, which are associated with race, but not gender (Ellard., 1984). The isoniazid AUC among persons who have fast acetylation is 30% to 50% of that among persons who have slow acetylation. Because isoniazid is well tolerated over a wide range of therapeutic doses, a single dose per body mass is recommended. Persons who have rapid acetylation achieve effective concentrations, while persons who have slow acetylation do not experience increased toxicity. Half-life ($t_{1/2}$) may vary from 1 hour in fast acetylators ($t_{1/2}$ < 90min) to 3 hours in slow

acetylators ($t_{1/2}$ >90min). The usual adult dose of isoniazid is 5 mg/kg given once daily, up to a maximum of 300 mg given once daily. Isoniazid decreases the clearance of some medications that are metabolized in the liver (particularly carbamazepine, phenytoin, and diazepam) (Baciewicz et al. 1985). However in the context of multidrug therapy including rifampin, these potential drug-drug interaction are of little significance because the effect of isoniazid is counteracted by the more potent opposing effect of rifampin (Kay et al. 1985). The total 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 it is uncommon (<0.2%) at conventional doses. The risk of peripheral neuritis increases for persons who are malnourished or predisposed to neuritis by other illnesses. Concomitant administration of pyridoxine (vitamin B_6) is recommended for these persons, and will be given to all patients in this trial. 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 and occurs in 10-20% of persons receiving isoniazid. However, idiosyncratic severe hepatic reactions are uncommon but are more likely in older persons (up to 2.3% hepatitis incidence in persons more than 50 years old), and may be life threatening. Daily consumption of alcohol increases the risk of isoniazid-associated hepatotoxicity by approximately 4-fold.

3.5 Pyrazinamide

Pyrazinamide is an analog of nicotinamide and has unique activity against *M. tuberculosis*, allowing the duration of treatment to be decreased from 9 months to 6 months (assuming a rifamycin is used throughout). The mechanism of action of pyrazinamide remains incompletely understood, but there is evidence that pyrazinamide inhibits *M. tuberculosis* trans-translation. Pyrazinamide is well absorbed from the gastrointestinal tract and widely distributed into all tissues (Ellard et al., 1987). Usual doses are 15-30 mg/kg/d, up to 2 gm/d. Peak serum concentrations of about 45 mcg/ml are achieved approximately 2-3 hours after a dose. Food and antacids do not significantly affect the absorption of pyrazinamide. The half-life of pyrazinamide is approximately 9-10 hours, and is prolonged in the presence of hepatic insufficiency (Lacroix et al., 1990). Pyrazinamide is metabolized to pyrazinoic acid by the hepatic microsomal enzyme pyrazinamide deamidase. Approximately 40% of a dose is recovered in the urine as pyrazinoic acid and an additional 4% is excreted in the urine as the unchanged parent drug (Ellard, 1969). The remaining drug is thought to be excreted in the bile. There are no known clinically significant drug-drug interactions involving pyrazinamide. The most frequent side effects are skin rash, gastrointestinal intolerance, hepatotoxicity (1.3%), arthralgias (1-7%), hyperuricemia due to blockade of urate excretion (up to 66%), and rarely acute gouty arthritis (Patel et al., 1995; Ormerod et al., 1996). These side effects are seldom dose-limiting. Asymptomatic elevations in serum uric acid are frequent, usually occur during the first or second month of treatment, and are self-limited and require no specific treatment (Zierski et al., 1980). Minor arthralgias also may occur during pyrazinamide treatment and can usually be treated with salicylates or non-steroidal inflammatory agents such as indomethacin while continuing the drug. The most common serious side effect of pyrazinamide is hepatotoxicity. In 2 randomized clinical trials the addition of pyrazinamide to RH did not increase the rates of hepatotoxicity above that seen with the latter 2 drugs alone (Zierski et al., 1980; Combs et al., 1990). However, 3 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 (Yee et al., 2003; Schaberg et al., 1996; Dossing et al., 1996). Hepatitis risk persists with prolonged use (Reves et al., 2014).

3.6 Ethambutol

Ethambutol is an ethylene derivative of butane that interferes with cell wall synthesis in mycobacteria; other bacteria are uniformly resistant to ethambutol. In the treatment of human TB, ethambutol is effective in preventing the emergence of drug resistant strains, although it has no sterilizing activity at clinically-tolerated doses (Kohno et al. 1992). Ethambutol is well absorbed from the gastrointestinal tract, reaching peak serum concentrations of 3-5 mcg/ml in normal volunteers 2-4 hours after a dose. Food slows absorption and decreases the peak serum concentration by 10-20%, but has no effect on the total systemic exposure. Antacids decrease both the peak serum concentration and AUC, and so should not be administered at the same time. Ethambutol is primarily eliminated by the kidneys as unchanged drug; the serum half-life averages 4 hours. Patients with renal insufficiency are prone to accumulation of the drug and the resultant toxicity. There are no known drug–drug interactions involving 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 (Patel et al., 1995). 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, as is the case in this study.

3.7 Vitamin B6 (Pyridoxine)

Pyridoxine (vitamin B6) is an essential vitamin involved in carbohydrate, fat, protein and brain amine metabolism. Isoniazid competitively inhibits the action of pyridoxine in these metabolic functions and also causes increased urinary excretion of pyridoxine (Snider, 1980). Chronic isoniazid administration can result in pyridoxine deficiency, which may manifest as acral paresthesias due to peripheral, predominantly sensory, axonal polyneuropathy. Isoniazid-associated peripheral neuropathy is dose related and occurs in less than 1% of persons receiving isoniazid at recommended doses (Blumberg et. al, 2003). It occurs more frequently in HIV-infected persons, malnourished individuals, persons with chronic renal failure or diabetes mellitus, pregnant and breastfeeding women, and persons with heavy chronic ethanol consumption. Pyridoxine 25 to 50 mg daily by mouth may be administered to prevent isoniazid-associated peripheral neuropathy.

4 OBJECTIVES

4.1 Primary:

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase, to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

4.2 Secondary:

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and store biospecimens from consenting participants for the purpose of future research on discovery and validation of TB biomarkers
- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among patients with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine.

5 STUDY DESIGN

This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.

As described in subsequent sections, participant safety will be maximized and risks will be minimized by frequent study visits for safety assessments, intensive microbiological monitoring for TB treatment failure and relapse, and periodic review of unfavorable outcome rates by a Data and Safety Monitoring Board.

6 STUDY POPULATION

This will be a multisite international study. Male and female participants who are age 12 or older and suspected to have pulmonary tuberculosis will be enrolled into the study.

Target enrollment is 2500 participants.

Pregnant or breast-feeding women will be excluded from the study because of uncertainties about the safety of rifapentine, moxifloxacin, and pyrazinamide in these groups. The sex, ethnicity, and socioeconomic background of study participants are expected to mirror those of the populations served by local tuberculosis clinics and the populations most affected by tuberculosis worldwide.

Co-enrollment in other therapeutic clinical trials is not allowed.

6.1 Subject Inclusion Criteria

Individuals must meet all of the following inclusion criteria in order to participate in this study:

A. Suspected pulmonary tuberculosis plus one or both of the following: a) at least one sputum specimen positive for acid-fast bacilli on smear microscopy OR b) at least one sputum specimen positive for *M. tuberculosis* by Xpert MTB/RIF testing, with semiquantitative result of 'medium' or 'high' and rifamycin resistance not detected.

B. Age twelve (12) years or older

C. A verifiable address or residence location that is readily accessible for visiting, and willingness to inform the study team of any change of address during the treatment and follow-up period.

D. Women of child-bearing potential who are not surgically sterilized must agree to practice a barrier method of contraception or abstain from heterosexual intercourse during study drug treatment.

E. Documentation of HIV infection status.

F. For HIV-positive individuals, CD4 T cell count greater than or equal to 100 cells/mm^3 based on testing performed at or within 30 days prior to screening.

G. Laboratory parameters done at or within 14 days prior to screening:

- Serum or plasma alanine aminotransferase (ALT) less than or equal to 3 times the upper limit of normal
- Serum or plasma total bilirubin less than or equal to 2.5 times the upper limit of normal

- Serum or plasma creatinine level less than or equal to 2 times the upper limit of normal
- Serum or plasma potassium level greater than or equal to 3.5 meq/L
- Hemoglobin level of 7.0 g/dL or greater
- Platelet count of 100,000/mm^3 or greater

H. For women of childbearing potential, a negative pregnancy test at or within seven (7) days prior to screening

I. Karnofsky score greater than or equal to 60

J. Written informed consent

6.2 Criteria for Exclusion from Enrollment

An individual meeting any of the following exclusion criteria at the time of enrollment or initiation of study drugs will be excluded from study participation:

A. Pregnant or breast-feeding

B. Unable to take oral medications

C. Previously enrolled in this study

D. Received any investigational drug in the past 3 months

E. More than five (5) days of treatment directed against active tuberculosis within 6 months preceding initiation of study drugs

F. More than five (5) days of systemic treatment with any one or more of the following drugs within 30 days preceding initiation of study drugs: isoniazid, rifampin, rifabutin, rifapentine, ethambutol, pyrazinamide, kanamycin, amikacin, streptomycin, capreomycin, moxifloxacin, levofloxacin, gatifloxacin, ofloxacin, ciprofloxacin, other fluoroquinolones, ethionamide, prothionamide, cycloserine, terizidone, para-aminosalicylic acid, linezolid, clofazimine, delamanid or bedaquiline

G. Known history of prolonged QT syndrome

H. Suspected or documented tuberculosis involving the central nervous system and/or bones and/or joints, and/or miliary tuberculosis and/or pericardial tuberculosis

I. Current or planned use within six months following enrollment of one or more of the following medications: HIV protease inhibitors, HIV integrase inhibitors, HIV entry and fusion inhibitors, HIV non-nucleoside reverse transcriptase inhibitors other than efavirenz, quinidine, procainamide, amiodarone, sotalol, disopyramide, ziprasidone, or terfenadine. Individuals who are currently taking efavirenz-based antiretroviral treatment or for whom initiation of efavirenz-based

antiretroviral treatment is planned within 17 weeks following enrollment may participate, as per Section 8.3.10.

J. Weight less than 40.0 kg

K. Known allergy or intolerance to any of the study medications

L. Individuals will be excluded from enrollment if, at the time of enrollment, their *M. tuberculosis* isolate is already known to be resistant to any one or more of the following: rifampin, isoniazid, pyrazinamide, ethambutol, or fluoroquinolones.

M. Other medical conditions, that, in the investigator's judgment, make study participation not in the individual's best interest.

N. Current or planned incarceration or other involuntary detention.

6.3 Criteria for Exclusion after Enrollment ('Late Exclusion')

Microbiological confirmation of drug-susceptible tuberculosis is not expected always to be available at the time of enrollment. Enrolled individuals who are subsequently determined to meet either of the following criteria will be classified as 'late exclusions' and study treatment will be discontinued:

A. Screening, baseline, and Week 2 study visit sputum cultures all fail to grow *M. tuberculosis*.

B. *M. tuberculosis* cultured or detected through molecular assays (Cepheid Xpert MTB/RIF or Hain MTBDR*plus* assays) from sputum obtained around the time of study entry is determined to be resistant to one or more of isoniazid, rifampin, or fluoroquinolones.

7 ENROLLMENT, RANDOMIZATION, AND MASKING PROCEDURES

7.1 Enrollment Procedures

Individuals who are sputum smear microscopy positive for acid-fast bacilli AND/OR have at least one sputum specimen positive for *M. tuberculosis* with a semiquantitative result of 'medium' or 'high' by Xpert MTB/RIF testing (with rifampin resistance not detected) will be invited to participate in this study. Interested individuals will be provided with information about the study including risks and potential benefits of all study procedures. If site staff are satisfied that the potential participant understands the information and the potential participant is willing, the potential participant will be asked to consent to participate in the study. Study-specific procedures will be initiated only after an individual has provided written informed consent.

7.2 Randomization

This will be a randomized trial. Randomization and treatment arm assignment will be computer-generated centrally by the TBTC Data and Coordinating Center. Randomization will be stratified by site, by the presence of cavitation on chest radiograph at baseline (since cavitation is associated with a decreased rate of microbiological response to TB treatment), and by HIV status (HIV-uninfected vs. HIV-infected with CD4 T cell count greater than or equal to 200 cells/mm^3 vs. HIV-infected with CD4 T cell count less than 200 cells/mm^3). Eligible participants (who meet all of the inclusion criteria and none of the exclusion criteria) will be randomly assigned in a 1:1:1 ratio to the study arms. Random assignment sequences will be generated in a way that limits the imbalance between arms within strata while also ensuring that the sequence is not predictable based on previous assignments.

Cavitation is defined as a gas-containing lucent space at least 1 cm in diameter within the lung parenchyma surrounded by an infiltrate or fibrotic wall greater than 1 mm thick seen on chest radiograph. Cavitation seen only on chest tomography (e.g. CT), if done, does not satisfy this definition. Cavities should be distinguished from pulmonary cysts, which are usually thin walled, well-marginated lesions.

8 STUDY PROCEDURES

8.1 Clinical Evaluations

Clinical evaluations will be performed in accordance with a detailed Manual of Operating Procedures (MOOP).

8.1.1 Interview for demographic and contact information

Participants will be interviewed for demographic information including place of birth and date of birth. Contact information will be obtained, including participant location of residence and participant phone number(s), and names and phone numbers of family members/friends who can be contacted by study staff in the event of emergency or if study staff are not able to locate the participant. Identifying and locating information will be maintained only at the site; it will not be entered into the study data base.

8.1.2 Obtaining a medical history

Participants will be interviewed to obtain medical information including tuberculosis signs and symptoms, prior tuberculosis history, other health conditions and treatments (including HIV infection), medicines used, and allergies to medicines.

8.1.3 Obtaining sputum specimens

Sputum may be spontaneously expectorated or induced by aerosol inhalation of sterile nebulized saline. Study sites will use their local procedure for sputum induction. Saliva should not be collected in place of a sputum specimen.

8.1.4 Performing visual acuity and color vision testing

Visual acuity will be tested using a Snellen-type chart. Color vision will also be tested. Results of this testing will not be collected centrally unless a new diagnosis (e.g. ≥ two lines on a Snellen-type chart, or loss of color vision) are discovered.

8.1.5 Symptom assessment

Participants will be asked if they have experienced any of the following within 14 days prior to enrollment or since the previous study visit: fevers, cough, rash, itching, jaundice, nausea, vomiting, diarrhea, loss of appetite, vision problems, numbness/tingling of extremities, joint pain. In addition, participants will be asked if they have had other symptoms not listed above, and if yes, then those other symptoms will be recorded and graded.

8.1.6 Assessment for adverse events

Participants will be asked about symptoms and signs, new medical diagnoses, and hospitalizations since the last study visit.

8.1.7 Chest Radiograph

A posteroanterior chest radiograph will be performed at screening and at the week 26 visit, regardless of treatment arm.

8.2 Concomitant Medications/Treatments

The use of all non-study drugs (including over-the-counter medications) from 14 days before starting study drugs through the end of study drug treatment will be monitored and recorded.

Antimicrobials with known antituberculosis activity (see MOOP) should not be used during study drug treatment, and any participant who receives more than five consecutive days of one or more of those medications will be classified as being on a non-study regimen. Antimicrobials without significant anti-tuberculosis activity may be prescribed for intercurrent infections at the discretion of the investigator and will be recorded on study forms.

Participants requiring the use of iron-containing supplements, antacids containing aluminum and/or magnesium, sucralfate, and/or antidiarrheals that contain kaolin should take study drugs at least four hours before or eight hours after ingesting those products in order to avoid impaired absorption of study drugs.

Rifamycins induce hepatic enzyme systems that are active in the metabolism of some other drugs. A prominent drug interaction of rifamycins is that involving hormonal contraceptives. Women of child-bearing potential (i.e. not surgically sterilized or not postmenopausal for more than one year) will be advised to use one or more of the following contraceptive methods while on study treatment: non-hormonal intrauterine device; barrier methods; abstinence from heterosexual vaginal intercourse.

Another medically important drug-drug interaction is that involving many antiretroviral drugs and the rifamycins, which are potent inducers of the cytochrome P450 system. Nonetheless, in accordance with World Health Organization guidance, antiretroviral therapy is recommended for HIV-infected study participants; a regimen comprised of efavirenz, tenofovir, plus lamivudine or emtricitabine is recommended for use in study participants based on the absence of known clinically significant drug-drug interactions between these antiretrovirals and the rifamycins. Efavirenz, nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors are allowed while a participant is receiving study treatment, provided that the criteria outlined in Section 8.3.10 are met. Guidance with respect to other antiretroviral agents will be provided in the MOOP and will be updated as new information on drug-drug interactions emerges.

Moxifloxacin has been shown to prolong the QT interval on the electrocardiogram in some patients. Study drugs should not be administered concomitantly with quinidine, procainamide, amiodarone, sotalol, disopyramide, ziprasidone, or terfenadine.

Strategies for management of other common drug-drug interactions will be described in the MOOP.

8.3 Laboratory Evaluations

8.3.1 Sputum mycobacterial culture

See Section 11.1 'Summary of Bacteriological Methods'.

8.3.2 Hematology

Blood will be drawn for a complete blood count that includes a white blood cell differential, hemoglobin, and platelet count. This will require approximately 5 mL of EDTA anticoagulated blood.

8.3.3 Biochemistry

Blood will be drawn for measurement of alanine aminotransferase (ALT; serum or plasma), and total bilirubin (serum or plasma); in addition at screening blood (serum or plasma) will be tested for creatinine, potassium, and albumin. This will require approximately 5 mL of blood.

8.3.4 HIV testing

Counseling prior to and following HIV testing, and reporting of HIV testing results will follow local guidelines and regulations at each site. For study purposes, HIV-1 infection is defined as a positive result using any licensed rapid HIV test or any licensed HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit. Confirmation of the initial study test result is required and must utilize a different method than the one used for the initial study assessment. The confirmatory study test must also be licensed and may include western blot, a second antibody test by a method other than the initial rapid HIV and/or E/CIA, HIV-antigen, or plasma HIV RNA viral load. Two or more HIV-1 RNA viral loads of >1,000 copies/mL are also acceptable as documentation of HIV infection. Negative study HIV testing does not require confirmatory testing. Note: the term 'licensed' refers to an FDA-approved kit or, for study sites located in countries other than the United States, a kit that has been certified or licensed by an oversight body within that country.

8.3.5 CD4 testing

Individuals identified as being HIV-infected will have CD4 T cell count testing performed unless the results are available for a test performed at or within 30 days prior to screening. This will require up to approximately 5 mL of blood.

8.3.6 HIV viral load testing

Individuals identified as being HIV-infected will have HIV viral load measured unless the results are available for a test at or within 30 days prior to screening. This will require approximately 5 ml of blood. Testing can be by any method used routinely at the study site as a standard of care test.

8.3.7 Pregnancy testing

Serum or urine testing methods are acceptable.

8.3.8 Diabetes screening

Hemoglobin A1C is the preferred test. If hemoglobin A1C testing is not available at the study site, then either fasting blood glucose (defined as no caloric intake for at least 8 hours) or random blood glucose can be measured.

8.3.9 Pharmacokinetic sampling: Tuberculosis Drugs

8.3.9.1 Overview

In the context of this overall study, two types of pharmacokinetic (PK) sampling will be used: 'sparse' sampling and 'intensive' sampling. The intensive sampling component will be performed among a convenience sample of patients at a few selected sites with capacity to perform this activity; the intensive PK component will be described in a separate protocol and a separate intensive PK informed consent document will be used. Individuals not undergoing intensive PK sampling will have sparse PK sampling, regardless of assigned study arm. Sparse PK is described below and is a component of the main study protocol. Blood specimens will be shipped to a designated laboratory in the U.S. or South Africa where concentrations of TB drugs and their metabolites will be measured.

8.3.9.2 Timing of sparse PK sampling during study participation for rifapentine and moxifloxacin

The minimum number of study drug doses prior to the sparse PK blood draw is 14 (including standard DOT and supplementary doses). The preferable timing of sparse PK sampling is at the week 2, 4or 8 visit (when sputum is also collected). However, if dosing schedule or other factors do not permit sampling at one of these study visits, then sampling may be performed any time after 14 study drug doses and no later than the week 8 study visit.

8.3.9.3 Schedule of sparse PK blood samples for rifapentine and moxifloxacin

All blood samples will be collected in reference to directly observed doses of intensive phase study drugs -- the reference dose of study drugs is preceded by three directly observed study drug doses given approximately 24 hours, 48 hours, and 72 hours prior. Two or three blood samples, 6 ml each, will be obtained over an 8 hour period as specified in the MOOP. The first timed blood specimen will be obtained 24 hours ± 1 hour after the most recent DOT study drug ingestion (defined as reference dose #1). After obtaining the first timed blood specimen, the reference dose of study drugs ingested (defined as reference dose #2) will be ingested. The second timed specimen should be obtained 25 to 40 minutes after ingestion of the reference dose #2 of study drugs. The third timed specimen should be obtained 5 to 8 hours after ingestion of the reference dose #2.

8.3.9.4 Administration of the Reference Dose of Study TB Drugs

The reference dose of study drugs should be taken by the participant in a manner consistent with the way the participant usually takes the study drugs relative to consumption of (i.e. eating or fasting) and type of food. The timing of food relative to the reference dose of study drugs and a brief description of any food and liquids will be

recorded. Participants should abstain from alcohol for 48 hours prior to the reference dose of study drugs and until after the last PK blood specimen is collected.

8.3.10 Pharmacokinetic sampling: Efavirenz

A secondary objective of TBTC Study 31 will investigate efavirenz pharmacokinetics in patients randomized to either of the rifapentine containing regimen arms of TBTC 31 (Investigational Regimen 2 and 3). Participants receiving efavirenz based ART will fall into one of two groups for PK evaluation: 1) HIV-infected participants who meet both of the following criteria a) on efavirenz for 30 days or more at the time of study enrollment and b) with viral load less than 200 copies/mL based on testing performed within 90 days prior to screening (Group 1); or 2) HIV-infected participants started on efavirenz after initiation but before or at week 8 of study tuberculosis drugs (Group 2).

Efavirenz plasma concentrations will be evaluated early in the study and in a limited number of participants to determine whether or not standard efavirenz dosing (600mg daily) results in adequate efavirenz exposure in the presence of daily 1200mg rifapentine treatment. An initial group of 31 participants from Group 1 will be enrolled. After the first 31 participants in Group 1 are enrolled, no further Group 1 participants will be permitted to enroll until data from these first 31 evaluable individuals are evaluated and proved to be clinically acceptable. Group 2 participants will not be allowed to enroll into the study until data from 31 evaluable individuals in Group 1 have been evaluated and deemed clinically acceptable, that is, there is no compelling evidence of lack of adequate exposure to efavirenz in Group 1 patients.

The efavirenz PK data by Group will be judged acceptable if we have evidence that >80% of participants have estimated efavirenz mid-dosing interval concentrations **≥**1 mg/L. Any patient that has a baseline (pre-TB treatment) efavirenz concentration <1mg/L will be deemed non-evaluable for the efavirenz pharmacokinetic study. Methods for estimating efavirenz pharmacokinetic parameters are provided in Section 13.5.5. Blood specimens will be shipped to a designated laboratory in the U.S. where concentrations of efavirenz will be measured.

Group 1: The first evaluation will be made when baseline, weeks 4 and 8 efavirenz concentrations are available for 31 evaluable participants; if ≤20 of the 31 have acceptable efavirenz concentrations at both Weeks 4 and 8, the team would consider this to be of concern. This rule was developed to have a high likelihood (95% or higher) of continuing to accrue participants if the true underlying rate of having acceptable efavirenz concentrations is greater than 80%. If accrual or shipping patterns warrant, then an interim analysis prior to assessment of 31 participants may be performed; in this case, an independent statistician who has not seen any PK data will develop the decision rule to protect the original PK study design and participant safety. The second evaluation will occur when 90 participants in Group 1 are enrolled.

Group 2: Once the efavirenz PK data have been evaluated in the first 31 patients from Group 1 and deemed clinically acceptable (that is, >20 of the 31 have acceptable efavirenz concentrations at both weeks 4 and 8) enrollment will open to patients in Group 2 (HIV-Infected participants starting on efavirenz early after initiation of study tuberculosis drugs). PK assessments will occur in a similar manner as in Group 1 participants with two phases of assessments (N=31 and 90 patients) except that sampling will occur approximately 4 and 8 weeks following initiation of EFV.

In addition, HIV-1 viral load measurements will be conducted 8 weeks after starting efavirenz and at study week 17. Stopping rules for numbers of patients with acceptable efavirenz concentrations in Group 2 will be the same as those for Group 1.

If stopping rules are met for unacceptable proportions of participants with acceptable efavirenz concentrations at any time (after 31 or after 90 patients in either Group), the TBTC 31 team may consider appropriate action based on study results, including whether to advise adjusting efavirenz dose or to exclude efavirenz use in subsequent study participants. If dose adjustment of efavirenz is recommended the step-wise PK evaluations will continue in a similar fashion as with the 600mg dose to again determine if acceptable efavirenz concentrations are maintained with the increased dose. Stopping rules for unacceptable proportions of patients with less than 1mg/L concentrations of efavirenz will be the same as with the 600mg dose.

8.3.10.1 Efavirenz Sampling Strategy

8.3.10.1.1. HIV-infected participants on efavirenz at the time of study enrollment (Group 1): Blood (approximately 8 ml) for measuring plasma efavirenz concentrations will be obtained at screening or at baseline (prior to initiation of study tuberculosis drugs) and additionally at weeks 4, 8 and 17 while on efavirenz. Blood will be drawn approximately 12 hours (no sooner than 10 hours after and no later than 24 hours) after the previous dose of efavirenz was ingested. The time of that efavirenz dose and the time of blood collection will be recorded. HIV viral load testing will also be performed at weeks 8 and 17.

8.3.10.1.2. HIV-infected participants started on efavirenz after initiation of study tuberculosis drugs (Group 2):
Blood (approximately 8 ml) for measuring plasma efavirenz concentrations will be obtained at about 4 and 8 weeks following initiation of efavirenz (to coincide with study visits to the extent possible). Blood should be drawn approximately 12 hours (no sooner than 10 hours after and no later than 24 hours) after the previous dose of efavirenz was ingested. The time of that efavirenz dose and the time of blood collection should be recorded. HIV viral load testing will be performed at 8 weeks following initiation of efavirenz (to coincide with study visits to the extent possible) and at study week 17.

8.3.11 Pharmacogenomic testing: Host Genetic Analysis

This study will evaluate human gene polymorphisms that may affect pharmacokinetics of tuberculosis drugs and antiretroviral drugs. Polymorphisms in SLCO1B1 (which may affect pharmacokinetics of rifamycins), NAT2 (which affects pharmacokinetics of isoniazid), cytochrome P450 isoenzyme 2B6 (CYP2B6) (which affects pharmacokinetics of efavirenz) and possibly other genes will be assessed. Data from these genetic assays may be used as covariates or for stratification in analyses of study endpoints. Blood (approximately 8.5 ml) will be collected once after enrollment and stored frozen as specified in the MOOP. Blood specimens will be shipped to a designated laboratory in the U.S. for analysis.

8.3.12 Storage *of M. tuberculosis* bacterial isolates

The *M. tuberculosis* bacterial isolates from screening and baseline, as well as isolates cultured from specimens obtained at or after week 17 will be stored. Isolates should be labeled with study identification number and collection date, and not with participant name. Isolates will be shipped to the CDC TB reference laboratory upon request. These isolates will be used for confirmatory drug susceptibility testing, for study endpoint assessment and for genetic characterization of *M. tuberculosis.*

8.3.13 Samples for future analyses to identify potential biomarkers of tuberculosis treatment response

Sputum, urine, and blood will be stored from all consenting participants for the purpose of future research to identify potential biomarkers of tuberculosis treatment response. Specimens collected for storage will be labeled with study identification number and collection date, and will not be labeled with participant name. Specimens will be centrally stored at Biomedical Research Institute, Rockville, MD, USA. Consenting patients will be asked to provide sputum, blood (approximately 8 ml blood per blood draw), and urine samples at 5 visits (baseline, week 2, week 4, week 8, and at end of treatment). Additionally, samples should also be collected if a) failure or relapse is suspected and evaluated at an unscheduled visit or a visit other than one of the 5 visits indicated above; and/or b) the participant voluntarily withdraws from the study. Participant consent for stored specimens will be part of the study Informed Consent Form which will be administered during the Screening/Consenting visit, with an independent signature line. As an alternative to the sample storage plan described above, participants enrolled in this clinical trial will be permitted to co-enroll in The Platform Study Biobanking Substudy (TBTC Study 36A), which includes additional sample types and additional timepoints for collection, requiring a separate TBTC Study 36A biobanking informed consent document. Biomarker sampling for a given participant will be only for Study 36A or for Study 31 but not for both.

8.4 Specimen preparation, handling and shipping

Specimen preparation and handling will be performed according to procedures set forth in the MOOP. Specimens will be labeled with study identification number and not with participant name. Shipping will be performed by trained personnel according to the Manual of Procedures and in accordance with local and international regulations.

8.5 Loss to follow-up

All efforts should be made to contact participants that miss DOT visits or scheduled study visits (unless the participant has withdrawn consent).

For participants who fail to attend a scheduled study visit during study treatment and attempts to reach the participant by phone are not successful or not feasible, a home visit will be performed within one week of the scheduled study visit.

For participants who fail to attend a study visit during study follow-up (off study treatment) and attempts to reach the participant by phone are not successful or not feasible, a home visit will be performed within approximately two weeks of the scheduled study visit. If a participant misses their final scheduled visit then repeated efforts must be made to contact the participant, until six weeks after the Month 18 visit of

the Last Participant In. If these attempts are unsuccessful then the participant should be considered lost to follow-up and reported as such.

8.6 Management of a participant with a positive sputum culture for *M. tuberculosis* at or after week 17 but before week 26

In the event of a positive culture confirmed as *M. tuberculosis* from a sputum specimen obtained at or after week 17 but before week 26, at least 3 repeat sputum samples should be obtained and sent for smear and culture. If *M. tuberculosis* is isolated in culture, drug susceptibility testing should be performed on one of the isolates.

The participant's case will be referred to the central study clinician for classification as either at risk for unfavorable outcome or not at risk for unfavorable outcome based on review of previous microbiology results and clinical information. Any participant determined to be at risk for an unfavorable outcome will be taken off of assigned study treatment and treated in accordance with local guidelines. The participant's treatment will not be changed without central study clinician consultation unless the site investigator determines that a change is needed for emergency circumstances. The central study clinician will be blinded with respect to treatment assignment.

8.7 Management of a participant with a positive sputum smear at or after week 17

In the event of a positive sputum smear from a specimen obtained at or after week 17, a repeat sputum sample should be obtained and sent for smear and culture. If *M. tuberculosis* is isolated in culture, drug susceptibility testing should be performed on one of the isolates.

Sputum smear results should not be used for clinical decision-making during the study. Participants with a positive sputum smear at or after week 17 will be referred to the central study clinician for further evaluation, including additional action if indicated, and follow-up, as outlined in section 8.6.

8.8 Management of a participant with a positive sputum culture for *M. tuberculosis* at or after week 26

In the event of a positive sputum culture for *M. tuberculosis* from a specimen obtained at or after week 26, a repeat sputum sample should be obtained and sent for smear and culture. If *M. tuberculosis* is isolated in culture, drug susceptibility testing should be performed on one of the isolates.

TB treatment change (including restarting treatment) is usually not indicated by a single positive culture, and is usually not considered unless there is worsening of clinical signs/symptoms and/or chest X-ray findings. If the participant remains well clinically (i.e. there is no clinical or radiological evidence of failure or relapse), then the clinician may consider requesting additional sputum samples and reviewing the participant's progress in two to four weeks depending on the clinical condition of the participant.

8.9 Management of a participant in whom a treatment change is being considered

If a treatment change is being considered, perform all of the following:

- Collect at least three additional sputum samples. These sputa should be sent to the study laboratory for smear and culture. If *M. tuberculosis* is isolated in culture, drug susceptibility testing should be performed on one of the isolates.

- Two (2) of these sputum specimens must be obtained prior to changing or re-initiating TB treatment and collected at least 4 hours apart.

- Discuss the proposed treatment change with the central study clinician before implementing the treatment change.

- For participants consenting to storage of specimens, obtain blood for plasma, sputum and urine for frozen storage.

Treatment extension or re-initiation of treatment in a participant should never be implemented prior to reviewing the participant's case with the central study clinician.

Treatment of participants in whom TB treatment is changed or re-initiated should be according to local guidelines.

8.10 Management of a participant who is discontinued from study treatment

8.10.1 Management of a participant who is discontinued from study treatment because s/he is determined to be ineligible after enrollment ('late exclusions')

These individuals should have study drugs discontinued, and undergo an early termination visit approximately 14 days after discontinuation of study drugs. The assessments to be performed at the early termination visit are in Section 9. Individuals should be referred to local sources of medical care.

8.10.2 Management of participants who become pregnant during the study

Women who become pregnant while receiving study therapy will be taken off of study treatment and treated according to National Tuberculosis Program or local guidelines. The women will continue to receive scheduled study follow-up, will be classified as being on a non-study regimen, and will not receive study radiographs. The outcome of the pregnancy will be reported on study forms.

Women who become pregnant while in study follow-up (not on study treatment) will continue to receive scheduled study follow-up, and will not receive study radiographs. The outcome of the pregnancy will be reported on study forms.

8.10.3 Management of participants who are discontinued from study treatment in the setting of an adverse event or the investigator judges that discontinuation of study treatment is in the participant's best interest

The site investigator may discontinue a participant from treatment in the event of a severe or serious adverse event, or at any time if the investigator thinks discontinuation is in the participant's best interest. Discontinuation from study treatment should be discussed with the central study clinician prior to discontinuation, unless discontinuation is considered an emergency by the site investigator. For study purposes, the participant should continue to be followed in the study per the study schedule and remain under the supervision of the site investigator, unless the participant withdraws consent. The participant should be referred to appropriate local sources of care for management of medical problems that cannot reasonably be managed by the study team.

8.10.4 Management of a participant who requests premature discontinuation from study treatment

A participant may request premature discontinuation from study treatment. An early termination visit should be performed and the participant should continue to be followed in the study per the study schedule, unless the participant withdraws consent. The participant should be referred to appropriate local sources of care for management of tuberculosis.

8.10.5 Management of a participant who is incarcerated after enrollment

This study will not enroll prisoners. However, it is possible that a participant will be incarcerated after enrollment. If an enrolled individual is incarcerated, then study medications will be stopped and the participant will be treated for active TB according to the standards of the institution in which s/he is incarcerated. While incarcerated, individuals will not be followed in the study. When the individual is no longer incarcerated, study treatment and/or study follow-up may continue, at the discretion of the investigator.

8.11 Premature termination of the study or closure of a study stratum or a study site

The study or a study stratum can be terminated by the sponsor on the advice of the Data and Safety Monitoring Board. The sponsor has the right to close the study and the sponsor has a right to close a site, although this should occur only after consultation between involved parties. In the event of study termination or site termination, the central and local ethics committees/institutional review boards must be informed. If the study or a study site is closed before the planned end of the study, all study materials (except documents required to be retained and stored on site) must be returned to the sponsor. The site investigator will retain all other documents until notification is given by the sponsor and/or as required by the local regulatory authorities. If the study or a study site is closed prematurely, participants should undergo an Early Termination Visit unless otherwise directed by the sponsor.

9 STUDY SCHEDULE

Activities to be conducted at study visits are also shown in Appendix A.

9.1 Screening

The following will be performed after written informed consent:

- Assessment of inclusion and exclusion criteria
- Interview for demographic and contact information
- Medical history
- Height and weight will be measured
- Chest radiograph unless results of a chest radiograph done within the previous fourteen (14) days are available.
- An HIV test will be obtained when any one or more of the following apply: a) HIV serostatus is unknown; b) the last documented negative HIV test was > 3 months prior to screening; c) written documentation of HIV-1 infection at any time prior to study entry is not available for confirmation of HIV-infected status.
- For individuals known or suspected to be HIV-infected, CD4 T cell enumeration should be performed unless the results are available for a test performed within 30 days prior to screening.
- For individuals known to be HIV-infected, an HIV viral load should be performed unless results are available for a test performed within 30 days prior to screening.
- Urine or serum pregnancy test for women of child-bearing potential, unless results of a negative are available for a test performed within seven (7) days prior to screening.
- A sputum sample will be obtained and sent to the study laboratory for mycobacterial smear and culture.
- Sputum for rapid molecular test, if available at site
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)
- Diabetes screening.
- Blood will be obtained for a complete blood count (including white blood cell differential, hemoglobin, and platelet count), and serum or plasma will be tested for alanine aminotransferase (ALT), total bilirubin, creatinine, albumin, and potassium, unless results from those tests performed within fourteen (7) days prior to screening are available.

9.2 Baseline

The baseline visit is defined as the visit at which study drug treatment is initiated. A baseline visit will be performed for individuals who meet study eligibility criteria and are willing to participate in the study. The

baseline visit should be performed as soon as possible and up to 7 days after screening. If applicable the screening and baseline activities may be performed in the same visit, with two sputa sent to the study laboratory. The baseline visit is defined as the visit during which study drug treatment is initiated. Participants who no longer meet eligibility criteria will be referred to a local source of tuberculosis care, and to other appropriate sources of clinical care as applicable. A log will be maintained of individuals who are screened but not enrolled.

The following will be performed at baseline:

- Review of interval tuberculosis treatment to assess whether the participant continues to meet eligibility criteria
- Review of concomitant medications to assess whether the participant continues to meet eligibility criteria
- Review of participant personal contact information
- Symptom assessment
- Concomitant medication assessment
- Measurement of weight
- Visual acuity and color perception testing
- A sputum sample will be obtained and sent to the study laboratory for smear and culture.
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)
- For participants consenting to storage of specimens for future identification of potential biomarkers of TB treatment response: a sputum specimen will be obtained and stored frozen; a urine specimen will be obtained and stored frozen; blood will be obtained for frozen storage of plasma.
- For HIV-infected participants on efavirenz and rifapentine treatment: plasma for efavirenz quantification
- Randomization

9.3 Study Visits at Weeks 2, 4, 8, and 12

Study visits should be performed within a window of +/- three (3) days. The following will be performed at each study visit:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- ONE SPUTUM obtained for mycobacterial smear and culture
- Measurement of weight

- In addition, at week 4 for all participants: testing of visual acuity and color perception should be performed.
- In addition at weeks 4 and 8 for HIV-infected participants on efavirenz: plasma for efavirenz quantification and HIV viral load testing at week 8
- For participants consenting to storage of specimens for future identification of potential biomarkers of TB treatment response: a sputum specimen will be obtained and stored frozen; a urine specimen will be obtained and stored frozen; blood will be obtained for frozen storage of plasma.
- In addition, any time after completion of 14 study drug doses and no later than the week 8 study visit for all participants: PK sampling for TB drug
- In addition, once any time after enrollment for all participants: blood for pharmacogenomic testing

9.4 Week 17

This visit should be performed within a window of +/- three (3 days). The following will be performed:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- TWO SPUTA will be obtained for mycobacterial smear and culture
- Measurement of weight
- For HIV-infected participants on efavirenz: plasma for efavirenz quantification, HIV viral load testing. For participants consenting to storage of specimens for future identification of potential biomarkers of TB treatment response: a sputum specimen will be obtained and stored frozen; a urine specimen will be obtained and stored frozen; blood will be obtained for frozen storage of plasma.
- For all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.5 Week 22

This visit should be performed within a window of +/- three (3) days. The following will be performed:

- Review of participant personal contact information

- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- TWO SPUTA obtained for mycobacterial smear and culture
- Measurement of weight
- Blood creatinine, ALT, total bilirubin, and complete blood count including WBC differential and platelets
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.6 Week 26

This visit should be performed within a window of +/- seven (7) days. The following will be performed:

- Review of participant personal contact information
- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- TWO SPUTA obtained for mycobacterial smear and culture
- Chest radiograph
- Measurement of weight
- For participants consenting to storage of specimens for future identification of potential biomarkers of TB treatment response who in addition have been assigned to the 26-week treatment arm: a sputum specimen will be obtained and stored frozen; a urine specimen will be obtained and stored frozen; blood will be obtained for frozen storage of plasma.
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.7 Study Visits At Months 9, 12, 15, and 18

Study visits should be performed at months 9, 12, 15, and 18. Visits should be performed within a window of +/- seven (7) days. The following will be performed at each study visit:

- Review of participant personal contact information
- Symptom assessment
- Review of interval medical history

- Concomitant medication assessment
- Measurement of weight
- TWO SPUTA obtained for mycobacterial smear and culture. For participants whose Month 18 sputa are both contaminated, two additional sputa should be obtained.
- In addition, for all participants (if not already done): blood for pharmacogenomics testing
- Storage of *M. tuberculosis* bacterial isolate (if culture positive)

9.8 Early Termination Visit for Participants Terminating Before Completion of their Assigned Study Treatment

The early termination visit should occur approximately 14 days after stopping study drugs, in order to assess for late manifesting adverse events. The following should be performed:

- Symptom assessment
- Adverse event assessment
- Concomitant medication assessment
- Blood creatinine, ALT, total bilirubin, and complete blood count (including WBC differential and platelets)
- One sputum obtained for mycobacterial smear and culture
- Chest radiograph
- Measurement of weight

Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event (see Section 12). Participants who were receiving study treatment at the time of early termination will be referred to local sources for tuberculosis care.

A participant who withdraws consent will not have to undergo follow-up study procedures.

9.9 Early Termination Visit for Participants Terminating After Completion of their Assigned Study Treatment

Procedures for visits at Month 9 should be followed. In addition, for participants consenting to storage of specimens for future identification of potential biomarkers of TB treatment response: a sputum specimen will be obtained and stored frozen; a urine specimen will be obtained and stored frozen; blood will be obtained for frozen storage of plasma.

Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event (see Section 12).

A participant who withdraws consent will not have to undergo follow-up study procedures.

9.10 Unscheduled Visit

Any visit conducted in addition to those required as per the study schedule will be considered to be unscheduled regardless of the reason for the visit. A sputum sample should be collected for mycobacterial culture if that participant is capable of producing sputum. Other assessments that are undertaken should be as clinically indicated.

9.11 Early/Late Visit

Any visit that a patient attends outside of the specified window but not in addition to those required as per the study schedule will be considered as an early/late visit and marked as such on study forms. Assessments undertaken should be those for the scheduled visit that has been missed or will be missed. Other assessments should be undertaken as clinically indicated.

10 STUDY INTERVENTION/INVESTIGATIONAL DRUGS

10.1 Study Drugs

The study drugs are rifampin, rifapentine, pyrazinamide, ethambutol, isoniazid, moxifloxacin, and pyridoxine (vitamin B6). Each of the study products is described in section 3.

The study products will be combined into the following study treatment regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

Rifampin, isoniazid, pyrazinamide, and ethambutol are the current first-line anti-tuberculosis drugs used worldwide for treatment of drug-susceptible tuberculosis. Pyridoxine (vitamin B6) decreases the risk of isoniazid-related peripheral neurotoxicity and is commonly co-administered with isoniazid during tuberculosis therapy. Each of these agents will be used at conventional doses and dosage schedules during this clinical trial.

Rifapentine, a rifamycin antibiotic, is approved in the United States by the Food and Drug Administration for the treatment of pulmonary tuberculosis; the approved dose is 600 mg twice weekly for two months followed by 600 mg once weekly for four months, in combination with other anti-tuberculosis drugs. As detailed above, pre-clinical and clinical studies indicate that maximal anti-tuberculosis activity is achieved at rifapentine doses higher than 600 mg and intervals shorter than twice weekly. During this clinical trial rifapentine will be administered at a dose of 1200 mg, once daily for 17 weeks in Regimens 2 and 3.

Moxifloxacin is a broad-spectrum fluoroquinolone antibiotic active against *M. tuberculosis*. It is used worldwide for treatment of drug-resistant tuberculosis. During this clinical trial moxifloxacin will be administered at the conventional adult dose of 400 mg once daily, and it will be administered for seventeen weeks in Regimen 3.

10.2 Study Drug Acquisition

All study drugs, except for vitamin B6 for the full duration of study therapy will be provided by Sanofi. Vitamin B6 supplies will be obtained locally. Study drugs will be distributed in bulk to study site pharmacies, which will dispense indicated supplies for individual participants according to individual pharmacy plans that will be approved by the sponsor or the sponsor's designee. Treatment of participants who discontinue study treatment will be with locally supplied drugs.

10.3 Study Drug Storage and Stability

Study drugs will be stored in accordance with the written MOOP, and may not be used after their expiration date.

10.4 Administration and Dosage of Study Drugs

All drugs will be administered orally, seven days per week, throughout treatment. Five of seven doses per week will be given as DOT by study personnel, or by a healthcare worker or lay treatment supervisor who is aware of the study protocol and trained regarding the study protocol. Doses on weekends and on holidays up to three consecutive days may be either DOT or self-administered. Participants receiving a rifapentine-containing investigational regimen should take study drugs within one hour after ingesting food. Participants receiving a rifampin-containing investigational regimen should take study drugs on an empty stomach; for participants on rifampin who have difficulty tolerating study drugs on an empty stomach, then administration with food is reasonable. Information about ingestion of food will be recorded for every study drug dose.

Doses of study medications will be determined by body weight, as follows:

Drug	Dose
Isoniazid	300 mg
Vitamin B6	25 or 50 mg
Pyrazinamide	
< 55 kg	1000 mg
≥ 55-75 kg	1500 mg
> 75 kg	2000 mg
Ethambutol	
< 55 kg	800 mg
≥ 55-75 kg	1200 mg
> 75 kg	1600 mg
Rifampin	600 mg
Rifapentine	1200 mg
Moxifloxacin	400 mg

Drugs and doses used to initiate treatment will be assigned by the enrollment application, based on weight reported at enrollment.

10.5 Dose Modifications for a Participant

10.5.1 Dose Modifications for a Change in Participant's Weight

Study drug doses for pyrazinamide and ethambutol should be adjusted for the participant's weight that was recorded at the most recent study visit.

10.5.2 Drug Re-challenges

In a participant experiencing side effects, whether to initiate a drug re-challenge as well as the drugs, dosages, and schedule for a drug re-challenge are at the discretion of the treating clinician. General recommendations for consideration are provided within the MOOP.

10.6 Criteria for Discontinuation of Study Drugs

Participants who meet any one or more of the following criteria will be discontinued from study treatment:

- Pregnancy
- Any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued administration of study treatment is not in the best interest of the participant
- Participant request for premature discontinuation of study treatment
- Participants classified as 'late exclusions'

Procedures for study follow-up are described in Section 9. Except in emergency situations, the decision to discontinue a participant from treatment should be first discussed with the central study clinician or his/her designee.

10.7 Accountability Procedures for the Study Drugs

Study drugs will be shipped periodically to the study sites. The investigator or designee will acknowledge receipt of and keep an inventory of study drugs. Unused product should be stored at the site until directed by the study sponsor.

10.8 Assessment of Subject Compliance with Study Treatment

Each dose of study drugs given by DOT will be recorded by the healthcare worker. Participants will be asked to record doses of study drugs not administered by DOT. Adherence will be reviewed at each study visit. Non-adherence should prompt an investigation into the cause of non-adherence and measures to address the non-adherence.

11 ASSESSMENT OF EFFICACY

11.1 Summary of Bacteriological Methods

Detailed laboratory procedures for laboratory staff performing mycobacteriological tests are in the MOOP. The following brief description is intended to provide guidance to clinical study staff.

All study sputa (expectorated or induced) should be sent to the designated study site laboratory. Any anticipated changes in the designated site laboratory (i.e. to a different laboratory) and/or a change in the type of solid or liquid media used must be communicated in writing to the TBTC Project Officer prior to executing the change(s).

The following will be performed for all study sputa: Nalc-NaOH decontamination followed by culture using both solid medium and the Becton Dickinson Mycobacterial Growth Indicator Tube (MGIT) liquid culture automated system. Bacterial load will be determined and recorded for all specimens using the time to positivity output automatically provided by the MGIT system.

For all cultures showing growth of acid-fast bacilli, the acid fast bacilli will be species identified at least to the level of *M. tuberculosis* complex vs. not *M. tuberculosis* complex.

For each participant, the first study isolate of *M. tuberculosis* will have culture-based phenotypic drug susceptibility testing performed at the study site-designated laboratory for isoniazid, rifampin, and fluoroquinolones. In addition, phenotypic drug susceptibility testing should be performed for the first of any *M. tuberculosis* isolates obtained at/after the Week 17 study visit.

For each participant, the first study isolate of *M. tuberculosis* as well as any *M. tuberculosis* isolates cultured from sputum obtained at/after the Week 17 study visit should be stored frozen. The first study isolate of *M. tuberculosis* should be sent to the CDC laboratory. Additional *M. tuberculosis* isolates pertinent to the participant's treatment assignment should be sent to the CDC laboratory if requested by the CDC Data Center.

12 ASSESSMENT OF SAFETY

12.1 Specification of Safety Parameters

12.1.1 Primary Outcome Measure

Proportion of participants with grade 3 or higher adverse events during study drug treatment

12.1.2 Secondary Outcome Measure

Discontinuation of assigned treatment for a reason other than microbiological ineligibility

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

12.2.1 Adverse Events and Serious Adverse Events

At each study visit participants will be asked about adverse events that have occurred since their previous visit. Solicited events will include symptoms, hospitalizations, and new medical diagnoses. Participants will also be asked to describe other signs, symptoms, and events not captured by the solicited event listing.

Monitoring of laboratory parameters will be performed as described in Section 9 and Appendix A.

12.2.1.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. 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, whether or not related to the medicinal (investigational) product.

Any adverse events detected will be graded according to the Common Toxicity Criteria for Adverse Events (CTC) toxicity tables and coded using MedDRA.

12.2.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse event that results in any of the following outcomes:

- Death
- Life-threatening (subject at immediate risk of death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect
- Results in a persistent or significant disability or incapacity
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate

medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

12.3 Recording and Reporting Procedures

12.3.1 Overview

A sign or symptom should be reported as an adverse event if it meets either (or both) of the following criteria: a) reaches severity of grade 3 or higher; b) represents a new diagnosis. Laboratory findings should be recorded as adverse events only if clinically significant.

12.3.2 Reporting Procedures

Any SAE or grade 4 toxicity must be reported by the site to the TBTC on an Adverse Event Report within 48 hours of the site's awareness of the event.

SAEs, death, or life-threatening problems *that may reasonably be regarded as caused by or associated with the administration of the study drug regimen* (defined as at least 'possibly associated' with the study drug regimen) must be reported by the site to the Sponsor (TBTC) and the local IRB within 48 hours of the site's awareness of the event. For participants who die, as much information as possible on cause of death and details of the final illness will be obtained from relevant sources.

Any adverse event that meets the definition of being a suspected unexpected serious adverse reaction (SUSAR) will be reported promptly by the Sponsor (TBTC) to the FDA, international regulatory agencies, the CDC IRB, and all clinical investigators. A SUSAR is defined as an adverse reaction that is unexpected (i.e. not consistent with the applicable product information) AND also meets the definition of a serious adverse event.

12.3.3 Pregnancy

For study purposes, pregnancy must be recorded on an Adverse Event Report form. Management of women who become pregnant during the study is described in Section 8.

12.4 Management of adverse events

For AEs that in the investigator's judgment may be due to study drugs, the following general approach to management should be applied. In general, for grade 1 toxicities, the patient will be followed carefully and the study drugs will be continued. For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs may be temporarily held if in the investigator's judgment continuation of study drugs would be unsafe.

Grade 3 and 4 AEs should be carefully assessed. The site clinician should consider other possible causes for toxicity before discontinuing study medicines. When possible, concomitant medicines should be withheld first at the discretion of the site clinician if it is suspected that concomitant medicines are contributing to the toxicity. If after careful assessment and in the site investigator's judgment the event is at least possibly attributable to study drug(s), then the

causative study drug(s) may be withheld; the participant should be permanently discontinued from study drugs if it is in their best interest.

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. Any participant for whom study drugs are temporarily held will be resumed on study medication as soon as possible.

12.4.1 Type and Duration of the Follow-up of Subjects After Adverse Events

Participants who experience adverse events that necessitate temporary or permanent discontinuation of study drugs will still be considered to be part of the study and will continue to be followed in the study until completion of follow-up. If study drugs are permanently discontinued, further anti-tuberculosis therapy may be administered at the clinic staff's discretion according to local or National Tuberculosis Program guidelines.

12.5 Data and Safety Monitoring Board

The TBTC Data and Safety Monitoring Board (DSMB) will review the study protocol and oversee progress of the trial. The TBTC DSMB is comprised of a clinical TB expert, an epidemiologist with extensive experience with TB and HIV, and an internationally recognized clinical trialist/statistician, all of whom are not otherwise involved in the study. The DSMB will convene approximately annually, or more often as needed.

12.6 Interim Monitoring and Analyses by the Data and Safety Monitoring Board

The DSMB will have access to data and interim results and may recommend early closure of any experimental arm or the trial if, in their judgment, interim evidence is sufficiently strong that one of the trial interventions is clearly indicated or clearly contraindicated because of a net difference in efficacy, safety, or tolerability as defined by the corresponding primary endpoints for the trial, with a difference between arms that is statistically significant at the 0.1% level, according to the Haybittle-Peto statistical criterion for monitoring interim efficacy data. The analysis sequence described in section 13.5 will be followed for interim analysis. It is possible that arm 3 could be inferior to arm 1 (e.g., due to less adherence, drug interactions), but arm 2 not inferior to arm 1. If this is occurs, the study team will discuss implications with the DSMB.

13 STATISTICAL CONSIDERATIONS

13.1 Study Hypotheses

This study has two main hypotheses and consists of two comparisons:

A) In previously untreated individuals with drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by nine weeks of rifapentine, isoniazid, and moxifloxacin (2PHZM/2PHM), all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with the standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid) all given daily throughout.

B) In previously untreated individuals with drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide, and ethambutol followed by nine weeks of rifapentine plus isoniazid (2PHZE/2PH), all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with the standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.

13.2 Study Outcome Measures

Primary Efficacy Endpoint:

- TB disease-free survival at twelve months after study treatment assignment

Primary Safety Endpoint:

- Proportion of participants with grade 3 or higher adverse events during study drug treatment

Secondary Efficacy Endpoints:

- TB disease-free survival at eighteen months after study treatment assignment
- Proportion of participants who are culture negative at eight weeks (solid and liquid media considered separately)
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated MGIT days to detection
- Sensitivity analyses assuming all losses to follow-up and non-tuberculosis deaths have an unfavorable outcome
- Sensitivity analyses assuming all losses to follow-up and non-tuberculosis deaths have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

13.3 Definition of primary outcome status

The primary analysis will be conducted using culture results from solid media and liquid media. Each participant will be classified into one of the following 3 outcome categories of Absence of Cure (Unfavorable Outcome), Cure (Favorable Outcome), or Not Assessable. An independent Endpoint Committee will be formed and be responsible for determining final outcome status. The primary efficacy endpoint will be assessed at 12 months after treatment assignment; a secondary efficacy endpoint will consider the follow-up period to be 18 months after treatment assignment.

Absence of Cure (Unfavorable Outcome) meeting any one or more of the following:

- Absence of bacteriological cure. A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17, that is culture positive for an *M. tuberculosis* strain that is indistinguishable from the initial isolate, and this is confirmed by a second sample that is culture positive for *M. tuberculosis*. A second confirmatory sample is required as a single positive sputum culture in isolation will not be considered absence of bacteriological cure.

- Participants who die from any cause during study treatment, except from violent or accidental cause (e.g. road traffic accident).

- Participants failing to complete treatment and not assessable at the end of the follow-up period.

- Participants who had a positive culture for *M. tuberculosis* when last seen, whether confirmed by a second sample or not, unless determined to have been re-infected

- Participants receiving any one or more of the following: a) extension of treatment beyond that permitted by the protocol; b) a re-start of treatment; c) a change in treatment for any reason except re-infection, pregnancy, or temporary drug challenge

Cure (Favorable Outcome) meeting any one of the following and not already classified as having an unfavorable outcome

- Participants with negative cultures at the end of the follow-up period

- Participants who at the end of the follow-up period are clinically without symptoms/signs of ongoing active TB and are unable to produce a sputum specimen

- Participants who at the end of the follow-up period are clinically without symptoms/signs of ongoing active TB and produce a sputum specimen that is contaminated without evidence of *M. tuberculosis*

Not Assessable (meeting any one or more of the following and not already classified as having an unfavorable outcome)

- Participants who completed assigned treatment and then default from follow-up, with their last culture being negative for *M. tuberculosis*

- Women who become pregnant during their assigned active treatment and stop their assigned treatment

- Participants who die during the follow-up phase (≥ 15 days after completion of study treatment)

- Participants who die from a violent (e.g. homicide) or accidental (e.g. road traffic) cause during their assigned active treatment. As above, suicide will be considered an unfavorable outcome

- Participants re-infected with a new strain of *M. tuberculosis,* demonstrated to be different from that identified at study entry through genotyping methods

13.4 Analysis Groups

There will be 4 analysis groups, as follows:

Intention-to-Treat (ITT)
Includes all enrolled participants who receive a treatment assignment.

Microbiologically Eligible
Includes the subset of Intention-to-Treat participants who, in addition, have culture confirmation of drug-susceptible tuberculosis at study entry. Participants classified as 'not assessable' will be considered to have an unfavorable outcome.

Assessable
Includes the subset of Microbiologically Eligible participants who, in addition, are not classified as 'not assessable'.

Adherent Per-Protocol
Includes the subset of Assessable participants who, in addition, complete assigned study treatment and follow-up.

13.5 Analysis Plan

13.5.1 Co-Primary efficacy analyses

There will be two co-primary efficacy analyses. One will consider the Microbiologically Eligible analysis population, and the other will consider the Assessable population. The assessable group is defined by exclusions that do not introduce selection bias. For each, the comparison of Regimen 1 (control regimen) versus Regimen 3 (2PHZM/2PHM) will be considered first, and, if non-inferiority criteria are met then the comparison of Regimen 1 versus Regimen 2 (2PHZE/2PH) will be considered. In each comparison, non-inferiority will be assessed by comparing the upper bound of a 95%, 2-sided confidence interval for the difference between the proportion of participants who are classified as having an unfavorable outcome on the control regimen (Regimen 1) and the intervention regimen to the predefined non-inferiority margin of 6.6%.

13.5.2 Primary safety analysis

There will be two comparisons: for comparison 1, Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH); for comparison 2, Regimen 1 (control regimen) will be compared against Regimen 3 (2PHZM/2PHM). The primary safety endpoint is proportion of participants with grade 3 or higher adverse events. The primary safety analysis will include the Intention-to-Treat group.

13.5.3 Secondary efficacy analyses

In secondary efficacy analyses, the primary efficacy endpoint will be assessed in the Adherent Per-Protocol analysis population, and secondary efficacy endpoints will be assessed for the Microbiologically Eligible, the Assessable, and the Adherent Per-Protocol analysis populations. Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH), and Regimen 1 will also be compared against Regimen 3 (2PHZM/2PHM).

13.5.4 Secondary safety analyses

Tolerability will be assessed as discontinuation of the assigned treatment for a reason other than microbiological ineligibility. Regimen 1 (control regimen) will be compared against Regimen 2 (2PHZE/2PH) and Regimen 1 will also be compared against Regimen 3 (2PHZM/2PHM). This analysis will include the Intention-to-Treat group.

13.5.5 Secondary Efavirenz PK analysis

Efavirenz pharmacokinetics will be evaluated in the first 31 and then a total of 90 patients from each of two groups of participants randomized to treatment regimen 2 or 3 of the study and receiving efavirenz based ART. Participants receiving efavirenz will fall into one of two groups for the PK evaluation: 1) HIV-infected participants on efavirenz with virologic suppression at the time of study enrollment (Group 1), or 2) HIV-infected participants started on efavirenz after initiation but before or at week 8 of study tuberculosis drugs (Group 2). Individual-level measurements of efavirenz mid-dosing interval concentrations and estimates of efavirenz apparent oral clearance using a model-based post-hoc Bayesian approach will be performed. The efavirenz PK data will be judged acceptable if we have evidence that >80% of participants have estimated efavirenz mid-dosing interval concentrations **≥**1 mg/L.

13.6 Sample Size Considerations

The primary objective of the trial is to evaluate whether rifapentine containing regimens can produce outcomes at least as favorable as standard therapy, but with a shorter treatment course. Therefore, the trial is structured as a non-inferiority study.

Key assumptions:

- Primary endpoint rate: 15% absence of cure (unfavorable) in the standard regimen arm (Microbiologically Eligible population). This rate is based on observed results for the control arm (MITT analysis group) in two recently completed phase 3 clinical trials (27/161 [14%] in the Rifaquin trial [Jindani et al., 2013] and 100/743 [13.5%] at 18 months post randomization and 114/679 [16.8%] at 24 months after the end of treatment in the Oflotub trial [Merle et al., 2013].)

- Margin to define inferiority: 6.6% (δ = 0.066)
- 95% confidence (type 1 error, α = 0.05). The sequential testing of regimen 3 and regimen 2 protects the type 1 error rate, as follows: If the statistical test for regimen 3 fails at 95% confidence, then conclude that both experimental regimens are not noninferior. If and only if regimen 3 is noninferior, then proceed to test regimen 2 at 95% confidence. A type 1 error occurs if either regimen is incorrectly deemed noninferior; the sequential approach limits the probability of this error to 5% overall.
- Power: 80% (type 2 error, β = 0.20) for primary analysis among Microbiologically Eligible subgroup, with power recalculated for the restriction to Assessable subgroup (see below)
- Proportion of enrolled patients who would be found to be late exclusions due to microbiological ineligibility – 12% (based on observed results in recent TBTC phase 2 studies)
- Proportion of enrolled patients who would be found to be 'not assessable': 12% (based on observed results in the Rifaquin trial [Jindani et al., 2013])

With 816 per arm, we expect 612 assessable. With the expected 15% unfavorable outcomes among those who are assessable, then with the same noninferiority margin and type 1 error rate, we have 90% power to test the primary hypotheses among the Assessable subgroup. The 6.6% margin to define inferiority (6.6%) takes into consideration the following issues: (1) the rates in historical trials of inpatient TB treatment for 6-month and 4-month regimens conducted by the British Medical Research Council support a difference in relapse up to 6% (East African/British Medical Research Council 1976, 1977, 1981; East and Central Africa/British Medical Research Council 1986; Singapore Tuberculosis Service/British Medical Research Council 1986; Nunn and Crook 2013); (2) recent trials in contemporary outpatient populations suggest a higher baseline proportion (15%) of unfavorable outcomes likely to be observed based on phase 3 trials and definitions; (3) the investigators in this trial and others perceive that the benefits of reducing treatment duration to 3 or 4 months would have advantages not outweighed by a possible increase in the relapse rate of up to 6%; and (4) the 6.6% margin does not imply that the experimental regimen may result in as much as 6.6% more unfavorable outcomes, but rather, for a fixed design, the maximum difference consistent with a non-inferior conclusion decreases as the proportion of unfavorable outcomes in the control arm increases.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Study staff will be trained in Good Clinical Practice and in performance of study procedures. The sponsor or the sponsor's delegate will conduct a meeting with each site prior to the trial opening at the site in order to ensure that everything is in place for the trial to start, the study file contains all the essential documents, and study staff understand procedures and their roles and responsibilities. The study will be conducted in accordance with the protocol and study-specific procedures manuals.

14.1 Local quality control

Each site will have a written plan for local quality control including data quality management.

14.2 External monitoring

External monitoring will be conducted to ensure the safety and conduct of this study, which involves patients with a serious illness (i.e. tuberculosis), uses an investigational drug treatment, will take place in multiple international sites, and may be used to modify the license for rifapentine and moxifloxacin.

External monitoring will be conducted periodically by a contract research organization (CRO). Direct access to data at each site will be required for the purposes of monitoring and audit, and this will be made explicit in the consent form. Local investigators and their institutions will provide direct access to source documents and data for trial-related monitoring, audit, and regulatory inspections, in the clinic, the pharmacy, and the mycobacteriology laboratory.

External monitoring will be conducted according to a written manual of procedures. Monitoring will focus on ensuring that the following study activities are conducted per the protocol and associated documents: consent procedures, enrollment, dispensing of trial medications, correct implementation of treatment and follow-up procedures, accurate recording and reporting of adverse events and appropriate reporting of adverse events, correct implementation and reporting of mycobacteriology laboratory testing, accurate completion of case report forms, and timely and accurate data entry. Additional activities or study elements may be monitored as needed.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

This study will be conducted in conformity with the ethical standards set out in the latest version of the Declaration of Helsinki.

15.1 Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents by an appropriate institutional ethics committee (IEC) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use.

15.2 Informed Consent Process

Adults: Only individuals who provide written informed consent will be enrolled in this study. Written informed consent is required before any study-specific procedures are performed. Potential participants will have the conditions of the study explained to them, including potential harms and benefits, the nature and timing of study procedures, alternatives to study participation, that study participation is voluntary, that a decision to not participate in the study will not affect the quality of their future medical care, and that they may withdraw from participation at any time. The information in the Informed Consent document will be translated into relevant local languages. Literate individuals will be provided with a language-appropriate document to read; illiterate individuals (i.e. individuals who speak and understand, but do not read and write, the language in which the consent discussion is conducted) will have the contents of the document explained to them by a trained study staff member; such individuals can be enrolled by 'making their mark' on the consent document. Potential participants will have the opportunity to ask questions of the site investigator or delegate, and to discuss participation with their family and/or friends or think about the study prior to deciding whether or not to participate. A copy of the signed informed consent document will be given to the participant for his/her records.

Children: For potential participants under 18 years of age, the assent of the child as well as the written informed consent of the child's legal guardian will be required for enrollment in this study. The child will receive, in language appropriate to the age and maturity of the child, an explanation of the research procedures; a description of the risks, discomforts, or inconveniences that the child might experience; and assurance that the child can withdraw from the study at any time. The assent process will be conducted by a study staff member who is experienced in consent and assent procedures, and in accordance with IRB/IEC requirements. All study participants age < 18 years will provide oral or written assent and written consent by the participant's legal guardian, in accordance with national and/or local IRB/IEC requirements.

15.3 Subject Confidentiality

All records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available without sufficient de-identification procedures.

Participant names will not be supplied to the sponsor. All study documents and forms will be identified by a code only. All paper study records will be stored in a locked office and electronic study records will be stored on password-protected computers; only designated trained study staff will have access to study records. Transmission of electronic records to the sponsor will occur through a web-based data entry application that conforms to the Federal Information Security Management Act, or using a secure CDC File Transfer Protocol (FTP).

The study monitors, other authorized representatives of the sponsor, and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records, primary laboratory data, and pharmacy records for study participants; this information will be provided to participants during the Informed Consent process. The clinical study site will permit access to such records.

15.4 Study Discontinuation

The Data and Safety Monitoring Board may recommend study termination or the termination of a study arm. In addition, after consultation with involved parties, the Sponsor has the right to close the study and the right to close a study site. If the study is closed, all involved ethics committees should be notified. If a site is closed, then the local IEC/IRB and the CDC IRB should be notified. In the event that the study is discontinued or a study site is closed, participants will undergo an early termination study visit. Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event. Participants who were receiving study treatment at the time of early termination will be referred to local sources for tuberculosis care.

16 DATA HANDLING AND RECORD KEEPING

ICH-GCP requirements for data documentation will be followed.

Data handling and record keeping will be performed in accordance with a study MOOP. Briefly, information from source documents will be recorded on study-specific case report forms, which will be entered into the study electronic information system. The study electronic information system will be programmed to maintain an audit trail, perform consistency checks, and generate reports of missing/inconsistent data. After study completion, de-identified data that can be legally released to the public may be released through a public-use data set after the data are evaluated for quality and confidentiality and shared with any partners, per CDC's policy on data sharing.

The study file and source documents should be retained until the sponsor gives notification.

17 PUBLICATIONS AND DISSEMINATION OF STUDY RESULTS

The study lead investigators and the TBTC Publications and Presentations committee must approve the use of any coded study data for the purposes of publication or presentation in advance. Any proposal for collection of additional data or analysis of study data must be agreed to in advance by the study lead investigators and the protocol team. The preparation of and authorship of publications arising from this study will be in accordance with the TBTC Bylaws.

These criteria will not apply to public-use data that have been made available in accordance with CDC's policy on data sharing. Persons who use publicly available data will be asked to acknowledge the TBTC and the S31 Protocol Team.

Updates on the progress of the trial will be presented at the twice-yearly TBTC meetings. Additional dissemination of results will be through the press, national governments at meetings and international organizations at conferences. Overall (aggregate) study results will be shared with study participants through mechanisms and materials reviewed and approved by the TBTC Community Research Advisors Group.

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SUPPLEMENTS/APPENDICES

Appendix A: Schedule of Procedures/Evaluations

Visit Window		Up to 7 days after screen	+/- three (3) days						+/- seven (7) days					Early Term. during tx.	Early Term. after tx.
Visit	**Screen**	**Baseline**	**WK 2**	**WK 4**	**WK 8**	**WK 12**	**WK 17**	**WK 22**	**WK 26**	**MO 9**	**MO 12**	**MO 15**	**MO 18**		
Informed Consent	X														
Inclusion/Exclusion	X	X													
Demographics	X														
Contact information	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Medical history	X														
Symptoms		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X					X	
Interval medical history										X	X	X	X		
Height	X														
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chest radiograph	X								X					X	
Visual tests		X		X											
HIV test	X														
CD4 T cell count (if HIV-pos)	X[a]														
HIV Viral load (if HIV-pos)	X[a]														
Pregnancy testing	X														
Randomization		X													
Sputum for smear and culture[b]	X	X	X	X	X	X	XX	XX	XX	XX	XX[c]	XX	XX	X	XX
Sputum for rapid molecular test, if available at site	X														
Storage of Mtb bacterial	X	X					X	X	X	X	X	X	X		

isolate															
Diabetes screen[d]	X														
ALT	X		X	X	X	X	X	X						X	
Bilirubin	X		X	X	X	X	X	X						X	
Creatinine	X		X	X	X	X	X	X						X	
Hemoglobin	X		X	X	X	X	X	X						X	
WBC with differential	X		X	X	X	X	X	X						X	
Platelets	X		X	X	X	X	X	X						X	
Albumin	X														
Potassium	X														
PK sampling for TB drugs			Obtain within this interval												
Blood sampling for pharmacogenomics testing		Obtain any time after enrollment													
Plasma for EFV PK and viral load (only for HIV-infected participants on RPT and efavirenz or starting[e] efavirenz		X			X		X								
Sputum, plasma, and urine specimens for storage (consenting participant only)		X	X	X	X		X[f]	X[f]							X

NOTES

[a] Unless results of a test performed at or within 30 days prior to screening are available.

[b] All sputa should be sent to the designated study laboratory with the exception of the screening specimen, which may be evaluated at any locally acceptable laboratory. Two specimens (i.e. one at screening and one at baseline) are required prior to initiation of study treatment. Two (2) sputa should be obtained at each of weeks 17, 22, 26 and at each of months 9, 12, 15, and 18.

[c] If both of the month 12 or month 18 sputa are contaminated, then the participant should be asked to provide two (2) additional sputa.

[d] Hemoglobin A1C is the preferred test. If such testing is not available, then fasting or random blood glucose can be measured.

[e] Collect 1st specimen at screening or baseline, not both. Participants starting efavirenz while on study TB treatment should have plasma obtained (for storage) within 7 days before starting efavirenz (window is immediately prior to 1st efavirenz dose to a maximum of 7 days prior to 1st efavirenz dose).

[f] To be collected at the end-of-study treatment visit (i.e. either week 17 or week 26).

Appendix B: Abbreviations

Abbreviation	Full-length identification
ACTG	AIDS Clinical Trials Group
AE	Adverse event
ALT	Alanine aminotransferase (alternative term is serum glutamic-pyruvic transaminase [SGPT])
AUC	Area under the concentration-time curve
CDC	Centers for Disease Control and Prevention (United States)
CFR	Code of Federal Regulations (United States)
CRO	Contract research organization
DOT	Directly observed therapy
DSMB	Data and Safety Monitoring Board
E	Ethambutol
EFV	Efavirenz
FDA	Food and Drug Administration (United States)
G	Gatifloxacin
GCP	Good Clinical Practice
H	Isoniazid
ICH	International Conference on Harmonisation
INH	Isoniazid
IEC	Institutional ethics committee
IRB	Institutional review board
ITT	Intention-to-treat
M	Moxifloxacin
MDR	Multidrug-resistant
MGIT	Mycobacterial Growth Indicator Tube
MIC	Minimum inhibitory concentration
MITT	Modified intention-to-treat
MOOP	Manual of Operating Procedures
MTB	Mycobacterium tuberculosis

P	Rifapentine
PD	Pharmacodynamics
PK	Pharmacokinetic
PP	Per-protocol
PZA	Pyrazinamide
R	Rifampin (alternative term is rifampicin)
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
TBTC	Tuberculosis Trials Consortium
WHO	World Health Organization
XDR	Extensively drug resistant
Z	Pyrazinamide

Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis:
A randomized, open-label, controlled phase 3 clinical trial

Consortium Identifiers:
Tuberculosis Trials Consortium Study 31
AIDS Clinical Trials Group A5349

ClinicalTrials.gov Identifier: NCT02410772

Statistical Analysis Plan

Version Number: 2.0
9 July 2019

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Approval:

Patrick Phillips, Trial Statistician

DocuSigned by:
Patrick Phillips
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7/10/2019

General Information

This document describes and substantiates the statistical principles and methods used for the analysis of data from TBTC S31/A5349. This document is designed to support the trial protocol. This Statistical Analysis Plan (SAP) supersedes previous versions of the SAP. Every care was taken in the drafting of this SAP, but corrections or amendments may be necessary.

Version 1.0 of the SAP was signed off prior to the database extract for the first interim efficacy analysis.

Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR, and applicable site-specific regulatory requirements.

Contents

1 Introduction

1.1 Protocol Summary

Title: Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: a randomized, open-label, controlled, phase 3 clinical trial

Hypotheses:	A) Seventeen (17) week rifapentine-based regimen In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine (P), isoniazid (H), pyrazinamide (Z) and ethambutol (E) followed by nine weeks of rifapentine plus isoniazid, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin (R), isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout. B) Seventeen (17) week rifapentine- plus moxifloxacin-containing regimen In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide and moxifloxacin (M), followed by nine weeks of rifapentine, isoniazid, and moxifloxacin, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.
Phase:	3
Design:	This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.
Population:	Participants with newly diagnosed, previously untreated pulmonary tuberculosis.
Number of Sites:	Multiple international sites, primarily sites of the Tuberculosis Trials Consortium and the AIDS Clinical Trials Group.
Study Duration:	Duration per participant is approximately 18 months.

Description of Agent or Intervention: After written informed consent, participants will be randomly assigned to receive one of the following oral regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

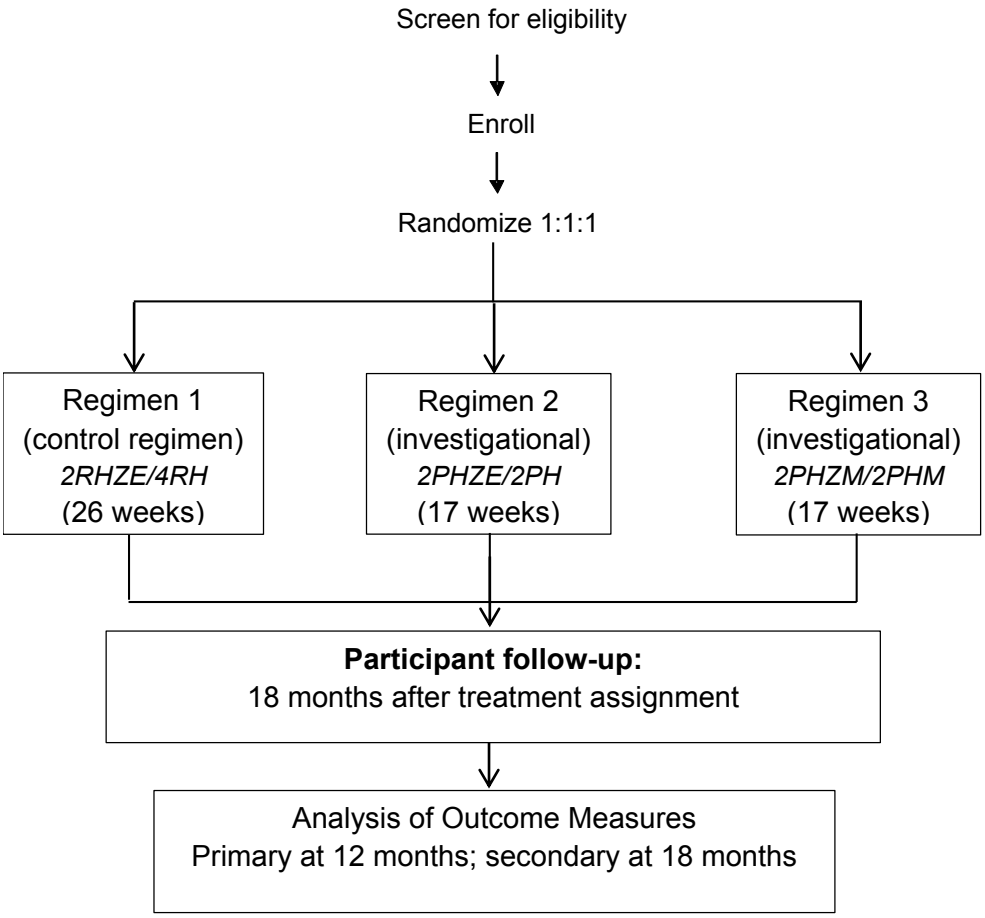
- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

1.2 Schematic of study design

Figure 1 Schematic of study design



1.3 Sample size considerations

The primary objective of the trial is to evaluate whether rifapentine containing regimens can produce outcomes at least as favorable as standard therapy, but with a shorter treatment course. Therefore, the trial is structured as a non-inferiority study.

Key assumptions:

- Primary endpoint rate: 15% absence of cure (unfavorable) in the standard regimen arm (Microbiologically Eligible population). This rate is based on observed results for the control arm (MITT analysis group) in two recently completed phase 3 clinical trials (27/161 [14%] in the Rifaquin trial[1] and 100/743 [13.5%] at 18 months post randomization and 114/679 [16.8%] at 24 months after the end of treatment in the Oflotub trial[2].)
- Margin to define inferiority: 6.6% ($\delta = 0.066$)
- 95% confidence (type 1 error, $\alpha = 0.05$). The sequential testing of regimen 3 and regimen 2 protects the type 1 error rate, as follows: If the statistical test for regimen 3 fails at 95% confidence, then conclude that both experimental regimens are not noninferior. If and only if regimen 3 is noninferior, then proceed to test regimen 2 at 95% confidence. A type 1 error occurs if either regimen is incorrectly deemed noninferior; the sequential approach limits the probability of this error to 5% overall.
- Power: 80% (type 2 error, $\beta = 0.20$) for primary analysis among Microbiologically Eligible subgroup, with power recalculated for the restriction to Assessable subgroup (see below)
- Proportion of enrolled participants who would be found to be late exclusions due to microbiological ineligibility – 12% (based on observed results in recent TBTC phase 2 studies)
- Proportion of enrolled participants who would be found to be 'not assessable': 12% (based on observed results in the Rifaquin trial[1])

With 816 per arm, we expect 612 assessable. With the expected 15% unfavorable outcomes among those who are assessable, then with the same noninferiority margin and type 1 error rate, we have 90% power to test the primary hypotheses among the Assessable subgroup.

1.4 Margin of non-inferiority

The 6.6% margin to define inferiority (6.6%) takes into consideration the following issues:

(1) the rates in historical trials of inpatient TB treatment for 6-month and 4-month regimens conducted by the British Medical Research Council support a difference in relapse up to 6% (East African/British Medical Research Council 1976, 1977, 1981; East and Central Africa/British Medical Research Council 1986; Singapore Tuberculosis Service/British Medical Research Council 1986; Nunn and Crook 2013);

(2) recent trials in contemporary outpatient populations suggest a higher baseline proportion (15%) of unfavorable outcomes likely to be observed based on phase 3 trials and definitions;

(3) the investigators in this trial and others perceive that the benefits of reducing treatment duration to 3 or 4 months would have advantages not outweighed by a possible increase in the relapse rate of up to 6%; and

(4) the 6.6% margin does not imply that the experimental regimen may result in as much as 6.6% more unfavorable outcomes, but rather, for a fixed design, the maximum difference consistent with a non-inferior conclusion decreases as the proportion of unfavorable outcomes in the control arm increases.

A 6% margin of non-inferiority trials has been used in other recent trials of single-drug substitution treatment shortening trials (e.g. REMoxTB). The justification of this margin is published in the online supplements with these papers (Gillespie et al, 2014 NEJM). We have attached the justification from that study as an attachment to support a 6% margin.

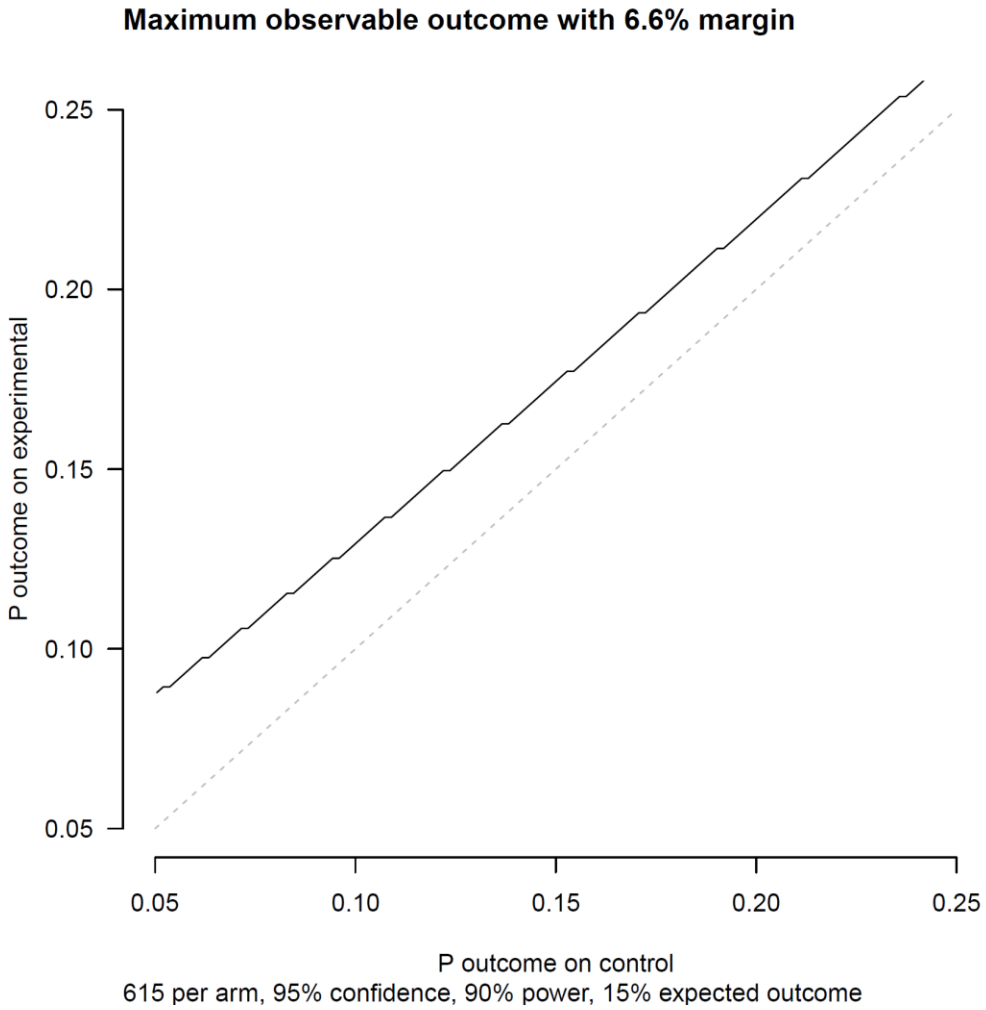
We believe an extension from 6% to 6.6% is justified for the following reasons:

1) The justification for a 4.8% margin in the FDA Guidance for Industry for Pulmonary Tuberculosis Trials is based on previous trials under 'per protocol' type analyses with many post-randomization exclusions, in a largely in-patient population; we observe larger proportions of unfavorable outcomes today than was seen in these relapse-only analyses from previous trials. We feel this provides a justification for a larger margin than 4.8%, and also the 6% that was justified for the REMoxTB trial. Recent trials in contemporary outpatient populations suggest a higher proportion (16% in REMoxTB MITT) of unfavorable outcomes, even than that anticipated in the REMoxTB trial sample size calculations (10%).

   Furthermore, the rationale for a 4.8% margin is based on the situation where a single drug that has an unknown contribution to the regimen is replaced by a new drug (the replacement of ethambutol, for example). In our study, rifampicin is replaced by rifapentine (in addition to the substitution of moxifloxacin for ethambutol in one arm). It is known that rifampicin is the most important drug in the current regimen. It might therefore be appropriate to consider not just the removal of the final two months of therapy (following the argument in lines 829-832 in the FDA Guidance) to estimate M_1, but also the consider the removal of rifampicin from the regimen. This would require consideration of a comparison of six months of HRZE (2HRZE/4HR) with four months of HZE (2HZE/2H) when estimating M_1. We are not aware of any trials that evaluated a 4-month regimen without rifampicin, so providing a comprehensive rationale similar to that which underpins the 4.8% would be challenging but would lead to a larger M_1 and therefore support a margin of non-inferiority larger than 4.8%.

2) Considering the clinical argument (from FDA Guidance and Nunn, Phillips, Gillespie 2008) we, and in broader consultation within our two large publicly-funded international consortia of TB stakeholders (CDC TB Trials Consortium and NIH AIDS Clinical Trials Group), consider the benefits of a 4-month rifapentine-based regimen justify the margin of 6.6%. Our consortia consider 600 patients per arm sufficiently large to provide adequate precision on the difference in efficacy between the regimens to determine whether an intervention regimen might be considered not inferior to the control regimen.

   The following graph was used to describe the maximum observable difference (solid line) from in the point estimate from the line of equality (plotted as dashed line) with a 6.6% margin under the stated assumptions.



The FDA Guidance for Industry Pulmonary Tuberculosis: Developing Drugs for Treatment identifies two studies comparing four and six months of TB therapy that provide data to estimate M1 for providing a rationale for the margin of non-inferiority. Study 2 (data from the 4th and 5th EA/BMRC trials) also included two four-month regimens without a rifamycin in the continuation phase, 2SHRZ/2HZ and 2SHRZ/2H. The combined relapse rate in these two arms was 63 (31%) / 203. Using the figures quoted in the FDA guidance document for the 2SHRZ/4HR regimen from this study (4.7% (8/172)), the treatment effect (4-month regimen minus 6-month regimen) is 26.4%, 95% CI (19.3%, 33.5%) for the unstratified risk difference. This lower bound of 19.3% provides an estimate of M1 for the removal of the final 2 months of HR therapy, and the removal of R in months 3 and 4. We want to preserve a reasonable proportion of this treatment effect and have therefore selected a 6.6% margin of non-inferiority which preserves more than 50% of M1.

For these reasons it is our perspective that a margin of 6.6% is justified.

2 Objectives

2.1 Primary Objectives

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase, to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

2.2 Secondary Objectives

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and assess biospecimens from consenting participants for the purpose of research on discovery and validation of TB biomarkers
- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among participants with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine.

3 Endpoints

3.1 Primary Endpoints

- Efficacy: TB disease-free survival at twelve months after study treatment assignment.
- Safety: Proportion of participants with grade 3 or higher adverse events during study drug treatment

3.2 Secondary Endpoints

- TB disease-free survival at eighteen months after study treatment assignment
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection
- Proportion of participants who are culture negative at completion of eight weeks of treatment (solid and liquid media considered separately)
- Sensitivity analyses assuming all participants classified as ‘not assessable’ have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

4 Study definitions

4.1 Definition of primary outcome

Each participant will be classified into one of the following three outcome categories:

1. Absence of Cure (Unfavorable Outcome)
2. Cure (Favorable Outcome), or
3. Not assessable.

The primary outcome is defined as twelve months after study treatment assignment. Actual visit dates, rather than scheduled visit names (e.g. Week 26, or Month 9), will be used for all analyses. See section 4.5 for visit windows that define the time periods. In particular, Month 12 includes data from visits up to 442 days from treatment initiation.

Only data up to the end of the Month 12 analysis visit window will be included in the primary analysis of the primary efficacy outcome.

4.1.1 Absence of Cure (Unfavorable)

A participant will be classified as having an unfavorable outcome if any one of the following conditions is met:

1. A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17 and no later than the end of the Month 12 analysis visit window, that is Mtb Culture Positive (see section 4.7 for definitions of culture results) that is indistinguishable from the initial isolate (see separate sequencing plan for definitions), and this is confirmed by a second sample that is Mtb culture positive. A second confirmatory sample, on a different day without an intervening Mtb Negative culture result, is required, as a single positive sputum culture result in isolation will not be considered absence of bacteriological cure. If results from strain analysis are inconclusive or unavailable, it will be assumed that strains were indistinguishable.
2. Participants who die from any cause during study treatment ('study treatment phase' is defined in section 4.6), except from violent or accidental cause (e.g. road traffic accident). Suicide during study treatment will be classified as an unfavorable outcome.
3. Participants who are withdrawn from follow-up or lost to follow-up prior to the scheduled end of treatment of study treatment, except for pregnancies and violent or accidental death that are instead classified as having a Not Assessable outcome (see section 4.1.3).
4. Participants who had an Mtb Positive culture result when last seen during or prior to the Month 12 analysis visit window, whether confirmed by a second sample or not, unless determined to have been re-infected.
5. Participants receiving any one or more of the following, except when given for failure or recurrence subsequently shown to be a reinfection with a strain of M. tuberculosis, different from that or those identified at study entry through genotyping methods):
    a) Extension of treatment beyond that permitted by the protocol; excepting
        a. Temporary drug re-challenge;
        b. Over-treatment with drugs from assigned study kits;
        c. Twenty-one days or fewer of non-study anti-TB medications given for treatment of active TB; or
        d. Secondary isoniazid preventative therapy in HIV infected participants.
    b) Re-start of treatment for active TB;
    c) Change in treatment (including frequency or dosage) for any reason except re-infection, pregnancy, or temporary drug challenge.
6. Participants who die during the follow-up phase (as defined in section 4.6) where the cause of death is considered related to tuberculosis.

4.1.2 **Cure (Favorable)**

A participant will be classified as having a favorable outcome if any one of the following conditions is met and an unfavorable outcome has not occurred:

1. Participants whose last culture result during the Month 12 analysis visit window is Mtb Negative (See Section 4.7).
2. Participants who are seen during the Month 12 analysis visit window and are clinically without symptoms/signs of ongoing active TB (indicated by absence of initiation of possible poor treatment response evaluation or PPTR that does not indicate presence of symptoms/signs of ongoing active TB), and have achieved culture conversion prior to Month 12, and
    1. Are unable to produce a sputum specimen at any point during the Month 12 analysis visit window; or
    2. Produce a sputum specimen that is contaminated or unevaluable without evidence of *M. tuberculosis,* and no sputum specimens yield positive or negative culture results during the Month 12 analysis visit window.

4.1.3 **Not Assessable**

A participant will be classified as having a Not Assessable outcome if any one of the following conditions is met and an unfavorable outcome has not occurred:

1. Participants not otherwise classified as unfavorable, but do not attend a visit within the Month 12 analysis visit window, and their last culture result is negative for *M. tuberculosis.*
2. Women who become pregnant during assigned study treatment (see section 4.6 for definition of study treatment phase).
3. Participants who die during the follow-up phase (as defined in section 4.6) of any cause that is not considered related to tuberculosis.
4. Participants who die from a violent (e.g. homicide) or accidental (e.g. road traffic) cause during their assigned study treatment (see section 4.6 for definition of study treatment phase). As above, suicide will be considered an unfavorable outcome.
5. Participants who are:
    a) Retreated, or have treatment changed or extended; and
    b) Demonstrated to be re-infected with a strain of M. tuberculosis, different from that or those identified at study entry through genotyping methods.

A participant classified as having a Not Assessable outcome will be excluded from the Assessable and Adherent Per-Protocol analyses, but considered as Unfavorable for other analyses.

4.2 **Participants Randomized in Error**

Participants who were randomized in error are those who were found to not meet eligibility criteria after enrollment, other than criteria in Section 4.3, relating to microbiology.

Determination of whether eligibility criteria was violated and subsequent classification as 'randomized in error' will be based only on data that was collected prior to randomization. All participants who are found to be in violation of any eligibility criteria (other than the criteria in Section 4.3 relating to microbiology) will be classified as randomized in error, irrespective of whether the participant was withdrawn from treatment or not.

4.3 **Exclusion criteria after enrollment ('Late Exclusion')**

Microbiological confirmation of drug-susceptible tuberculosis is not expected always to be available at the time of enrollment. Enrolled individuals who are subsequently determined to

meet either of the following criteria will be classified as ‘late exclusions’ and study treatment will be discontinued:

1. None of screening, baseline, and Week 2 study visit sputum cultures are Mtb Positive
2. *M. tuberculosis* cultured or detected through molecular assays (Cepheid Xpert MTB/RIF or Hain MTBDR*plus* assays) from any sputum obtained at screening, baseline, or week two study visit is determined subsequently to be resistant to one or more of isoniazid, rifampin, or fluoroquinolones.

4.4 **Adequate treatment**

Only participants having completed an adequate number of study doses will be included in the Per Protocol (PP) analysis populations. Two PP analysis populations are defined. PP75 excludes participants who have received less than approximately 75% of study doses (see Table below for exact doses required) using the definitions consistent with previous phase III TB trials, in particular the REMoxTB trial[3] and in the original trials which determined the effectiveness of the control 6 month isoniazid-rifampin regimen[4]. The TB-REFLECT analyses[5] have shown that even participants with less than 95% adherence have poorer outcomes than those with perfect adherence, and consecutive missed doses is associated with poorer outcomes than occasional missed doses[6]. For these reasons, the PP95 analysis population excludes participants who have received less than approximately 95% of study doses (see Table 1 below for exact doses required). PP95 will be the primary per protocol analysis population with PP75 being supportive.

Table 1. Two definitions of adequate treatment

	75% Adherence (PP75) Approximately 75% of doses within 125% of the intended duration.		95% Adherence (PP95) Approximately 95% of doses within 125% of the intended duration.	
	Doses	Days	Doses	Days since treatment initiation
Regimen 1	At least 42 intensive phase doses	No more than 70 days since treatment initiation	At least 54 intensive phase doses	No more than 70 days since treatment initiation
	At least 84 continuation phase doses	No more than 168 days since completing intensive phase	At least 120 continuation phase doses	No more than 168 days since completing intensive phase
	No more than 42 doses missed		No more than 5 consecutive DOT doses missed	
Regimens 2 or 3	At least 42 intensive phase doses	No more than 70 days since treatment initiation	At least 54 intensive phase doses	No more than 70 days since treatment initiation
	At least 42 continuation phase doses	No more than 84 days since completing intensive phase	At least 60 continuation phase doses	No more than 84 days since completing intensive phase
	No more than 28 doses missed		No more than 5 consecutive DOT doses missed	

4.5 Visit windows

Participants are assessed at screening, baseline (Week 0), Week 2, Week 4, Week 8, Week 12, Week 17, Week 22, Week 26, Month 9 and 3-monthly to Month 18 with visit dates scheduled from the date of treatment initiation.

For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of first dose of study medication. The only exception is the visit window for Baseline which is from date of screening consent to date of randomization (inclusive). Any visits after randomization up to 24 days after treatment initiation are included in the Week 2 visit window.

When referring to a visit in this analysis plan (e.g. 'Week 17'), this implies within the defined visit window as specified below in Table 2.

Table 2. Analysis visit windows

Visit	Target date (days from date of first dose of treatment)	Analysis window
Baseline (including screening period)	Date of randomization	Date of screening consent – Date of randomization
Week 2	14	Day after randomization-24
Week 4	28	25-52
Week 8	56	53-80
Week 12	84	81-115
Week 17	119	116-150
Week 22	154	151-178
Week 26	182	179-262
Month 9	270	263-352
Month 12	360	353-442
Month 15	450	443-523
Month 18	540	533-no upper bound (an upper bound of 570 will be used for reporting safety analyses)

Any visit, scheduled or unscheduled (including possible poor treatment response or post early termination visits), that falls into the analysis window will be assigned to that visit for the purpose of analysis. If two visits fall within the same interval, the one closest to the target date will be used for analyses by visit, or the highest value, depending on the analysis, so that there is only one unique visit for each participant and analysis time-point. However, all critical endpoint determining data will be used (e.g. culture results).

4.6 Treatment and follow-up phases

For the purposes of analysis, the screening, treatment and follow-up phases are defined as follows:

- **Pre-treatment phase**
  - Start: date informed consent signed.
  - End: day before start of study treatment.

- **Study treatment phase**
  - Start: date of start of study treatment.
  - End: date of last dose of allocated treatment regimen, plus 14 days.

- **Follow-up phase**
  - Start: the day after the end of the study treatment phase.
  - End: date of the last participant contact (scheduled or unscheduled, or other contact e.g. phone call).

4.7 Microbiologic classification

4.7.1 Inoculation results

Each sputum specimen is inoculated onto solid and liquid media. The result of each inoculation is classified as Mtb Positive, Mtb Negative, Contaminated, or Unevaluable (Table 3). It is expected that one sputum specimen will produce two inoculation results, one for each media type. Inoculation results reported as Mtb alone, Mtb with other mycobacteria (e.g., NTM), or Mtb with contamination will be classified as Mtb Positive. Inoculations reported as no growth or other mycobacteria without identification of Mtb will be classified as Mtb Negative. Inoculations reported as contaminated without identification of Mtb will be classified as Contaminated.

Table 3. Classification of culture results per inoculum

Inoculation Result Classification	**Definition**
Mtb Positive	**Definitive identification of Mtb with or without the presence of contaminants or other mycobacteria.**
Mtb Negative	**No Mtb or contaminants detected at least 42 days after inoculation. Growth of other mycobacteria (NTM) in the absence of Mtb or contaminants.**
Contaminated	**Growth of contaminants with or without other mycobacteria, but with no identification of Mtb.**
Unevaluable	**Includes any of the following:** • Sputum processed at non-lab of record. • Media inoculated more than 4 days after sputum received in the lab. • Missing culture result. • Mtb identification was presumptive with no definitive identification. • Result of no growth determined fewer than 42 days after inoculation.

4.7.2 Culture results

The primary analysis will be conducted using results from inoculation onto solid and liquid media. For each sputum specimen, results from solid and liquid media inoculations will be combined into a single Culture Result, see Table 4. When multiple sputa are collected on a single day, all results of each inoculation are used to establish the Culture Result.

For some analyses, solid and liquid culture will be analyzed separately. For these analyses, definitions in Table 4 will be used, considering only inoculations from that culture media.
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Table 4. Specimen Culture Result Classification for all inoculation results

Culture Result Classification	**Definition for visits when a single specimen (multiple media) is collected**	**Definition for visits when multiple specimens (multiple media) are collected**
Mtb Culture Positive	**Any inoculation from a single specimen is Mtb Positive**	**Any inoculation from any specimen collected on a single day is Mtb Positive**
Mtb Culture Negative	**All inoculations are Mtb Negative for a single specimen** **When at least one inoculation from a specimen is Mtb Negative and additional inoculation(s) is Contaminated** When at least one inoculation from a specimen is Mtb Negative and additional inoculation(s) is Unevaluable	**All inoculations from all specimens collected on a single day are Mtb Negative** **When at least one inoculation from all specimens collected on a single day is Mtb Negative and additional inoculation(s) is Unevaluable or Contaminated**
Culture Contaminated	**All inoculation results are Contaminated for a single specimen** When at least one inoculation from a single specimen is Contaminated and additional inoculation(s) is Unevaluable	**All inoculations from all specimens collected on a single day are Contaminated** **When at least one inoculation from all specimens collected on a single day is Contaminated and additional inoculation(s) is Unevaluable**
Culture Unevaluable	**All inoculation results from a single specimen are Unevaluable**	**All inoculations from all specimens collected on a single day are Unevaluable**
Unable to produce sputum	**Attempt to collect sputum is unsuccessful as participant is unable to produce sputum**	**All attempts to collect sputum is unsuccessful as participant is unable to produce sputum**

5 Efficacy Analyses

5.1 Study Populations

There will be five analysis groups, as follows:

5.1.1 Intention-to-Treat (ITT)

Includes all randomized participants.

5.1.2 Microbiologically Eligible

Includes the subset of Intention-to-Treat participants who, in addition, have culture confirmation of drug-susceptible tuberculosis as defined by section 4.3, and were not randomized in error as defined by section 4.2. Participants classified as 'not assessable' will be considered to have an unfavorable outcome in this analysis.

5.1.3 Assessable

Includes the subset of Microbiologically Eligible participants who, in addition, are not classified as 'not assessable'.

5.1.4 Adherent Per-Protocol (PP95)

Includes the subset of Assessable participants who, receive 95% of assigned treatment as defined in Section 4.4. Participants in the Assessable study population that do not complete adequate treatment for the reason of death or bacteriological treatment failure will be included in the PP95 analysis population provided they receive 95% of doses up to the time of treatment withdrawal. This will be the primary PP analysis population.

5.1.5 Adherent Per-Protocol (PP75)

Includes the subset of Assessable participants who, receive 75% of assigned treatment as defined in Section 4.4. Participants in the Assessable study population that do not complete adequate treatment for the reason of death or bacteriological treatment failure will be included in the PP75 analysis population provided they receive 75% of doses up to the time of treatment withdrawal. This is a supportive PP analysis population and is included for comparability with previous trials (particularly REMoxTB)

5.2 Analysis plan for the primary efficacy outcome

5.2.1 Co-primary efficacy analyses

The primary analyses of efficacy is based on the modified ITT populations: the Microbiologically Eligible and Assessable study populations. While an ITT analysis is commonly used for a superiority trial, it may not be sufficiently conservative for a non-inferiority trial since ITT analysis can bias toward no treatment difference. Both the International Conference on Harmonisation (ICH) E9 document of Statistical Principles for Clinical Trials[7] and the FDA document of Guidance for Industry Non-Inferiority Clinical Trials (REF) suggest looking at the ITT analysis cautiously for a non-inferiority trial and recommend considering PP analysis as equally important for a non-inferiority trial[8]. A PP analysis, however, does introduce bias through exclusions based on post-randomization exclusions[9] which is particularly problematic in a study that is not placebo-controlled. The PP analyses (PP95 and PP75) will therefore be considered secondary in the primary efficacy analyses.

There will be two co-primary efficacy analyses, one for the Microbiologically Eligible population and another for Assessable population. Non-inferiority must be demonstrated in both populations in order to declare non-inferiority for an intervention regimen.

For each, the comparison of unfavorable outcome as defined by protocol at primary efficacy endpoint for Regimen 1 (control regimen) versus Regimen 3 (2PHZM/2PHM) will be considered first, and, if non-inferiority criteria are met, then the comparison of Regimen 1 versus Regimen 2 (2PHZE/2PH) will be considered.
In the event that the arm of Regimen 3 is terminated for safety concerns in the interim monitoring, the comparison of Regimen1 versus Regimen 2 (2PHZE/2PH) will be carried out for co-primary efficacy analyses.
If Regimen 3 is not terminated for safety concerns in the interim monitoring and the non-inferiority criteria for Regimen 1 versus Regimen 3 at the primary endpoint are not met (see 5.2.1), then the comparison of Regimen 1 versus Regimen 2 at the primary endpoint will be considered as an exploratory efficacy analysis.

5.2.2 **Non-inferiority test**

The proportion of participants with unfavorable outcome can be estimated thus: if r_a and r_b are the proportions of unfavorable outcome for the two study arms (*a* = Regimen 1 and *b* = Regimen 2 or Regimen 3) and if δ is the non-inferiority margin (= 6.6%), then the statistical hypothesis is:

$$H_0 : r_b - r_a \geq \delta \quad \text{vs.} \quad H_1 : r_b - r_a < \delta$$

For a significance level of 0.05, the statistical test is equivalent to obtaining the **two-sided 95% confidence interval for the difference of $r_b - r_a$.** If the upper bound of the confidence interval is less than δ, then the non-inferiority of Regimen 2 or Regimen 3 to Regimen 1 with a significance level of 0.05 is established (equivalent to one-sided α=0.025).
The difference in proportion unfavorable will be calculated using a stratified analysis using Cochran-Mantel-Haenszel weights[10]. Randomization is stratified by site, HIV status and presence of cavitation. The analysis will be stratified by HIV status and presence of cavitation only. Inclusion of site as a factor in the stratified analysis will result in some groups with too few participants. The unstratified difference in proportions will also be presented, although the stratified difference will be considered primary.

5.2.3 **Superiority test**

Regimens that are shown to be non-inferior to control will also be assessed for superiority compared to control using a two-sided significance level of 0.05.

5.2.4 **Comparison of intervention arms**

If the intervention regimen 3 (containing moxifloxacin and rifapentine) is shown to be non-inferior to control, the two intervention regimens will be compared to each other in the primary efficacy outcome to assess the added effect of moxifloxacin to the regimen in a superiority comparison using a two-sided significance level of 0.05. This is an exploratory comparison as the study is not powered for this comparison.

5.2.5 **Secondary efficacy analyses**

In additional secondary efficacy analyses, the primary efficacy endpoint will be assessed in the PP95 and PP75 populations, in addition to the full ITT population.

5.2.6 Tabulation of the primary outcome classification

Since the primary efficacy outcome is a composite of various components, the actual reason (component) for outcome will also be tabulated by treatment arm.
Participants will be classified by the first event (chronologically) that made the participant unfavorable and further sub-classified by the bacteriological status at the time that this outcome occurred (culture converted, never culture converted, bacteriological reversion, or bacteriological relapse).

5.2.7 Sub-group analyses

This primary efficacy analysis will be repeated in subgroups according to the following baseline factors (i.e. those present at enrollment or from study-specific samples collected for screening and baseline visits). For factors reliant on results from sputum samples, the results must be from the study laboratory of record. Categorical variables will be split by tertiles except where there is previous clinical justification for a different cut-off.

- HIV status
- Presence of cavitation on baseline chest radiograph
- Extent of cavitation on baseline chest radiograph
- Sex
- Weight
- BMI
- WHO scale smear quantification
- Solid culture colony count
- MGIT days to detection
- GeneXpert MTB/RIF Cycle Threshold
- Age
- Country of study center
- Smoking history
- History of diabetes
- Ethnicity and race

The test for an interaction between the covariate and treatment will be done using logistic regression comparing the model including the interaction term and the model with only marginal terms using the likelihood ratio test to evaluate the statistical significance of inclusion of the interaction term in the model.

5.2.8 Independent Endpoint Review Committee

An independent blinded endpoint review committee (ERC) will be formed to review individual primary efficacy outcome classification at the end of the trial in the event that the assessment of non-inferiority is marginal for one or both regimens.
Members of the ERC will be identified prior to the end of the trial, and will include a small number of experts with experience in conducting clinical trials for new treatments for TB who are not otherwise involved in the trial. The ERC may not be needed, but may be convened and asked to review individual primary efficacy outcome classification for a subset of patients under the following scenarios:

- if the upper bound of the 95% confidence interval of the difference in the proportion of unfavorable outcomes is within 2% of the non-inferiority margin of 6.6%, in other words between 4.6% and 8.6%, for either regimen as compared to control provided the regimen is shown to safe and well tolerated. This range includes scenarios when non-inferiority has been demonstrated (<6.6%) or just missed (>6.6%).

- if there are large number of outcomes (10% or more) that are based on limited bacteriology or limited data and the review by the ERC would be considered valuable in interpreting the results.

The ERC may be asked to review outcome classification in other situations.
The ERC will review the full bacteriological, clinical and baseline data for participants, while staying blinded to treatment allocation. The ERC will review all participants classified as unfavorable or not assessable in the primary efficacy outcome, although they would be expected to focus on the more complex cases and more straightforward cases (e.g. clear bacteriological relapse) might be presented in summary form. The ERC will be asked to determine whether, based on the entirety of the data available, they would consider this participant to have had an unfavorable outcome of treatment or be in the process of having an unfavorable outcome of treatment and to quantify their confidence using a scale from 1 (very unlikely) to 4 (very likely). The ERC will be encouraged to come to consensus in each case. Any re-classification by the ERC will not replace the primary efficacy outcome defined based on the algorithm described in this analysis plan, but will be a sensitivity analysis used to supplement the primary efficacy results and provide further insight into whether the intervention regimens should be considered to have non-inferior efficacy.

A document further describing the terms of reference of the endpoint review committee will be prepared prior to the end of the trial.

5.2.9 **Additional sensitivity analyses**

The following additional sensitivity analyses will be conducted:

1. The primary efficacy analysis will be repeated in the Microbiologically Eligible study population where all participants classified as not assessable will be classified as favorable rather than unfavorable.
2. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations where participants taking any non-study anti-TB medications for more than 21 days for any reason (including secondary isoniazid preventative therapy) will instead be classified as unfavorable.
3. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations where participants taking any non-study anti-TB medications for more than 5 days for any reason (including secondary isoniazid preventative therapy) will instead be classified as unfavorable.
4. The primary efficacy analysis will be repeated with a modification to the definitions of 'Absence of Cure' using the following text to replace the paragraph numbered 1 in section 4.1.1. so that intervening negative cultures are ignored in the determination of absence of bacteriological cure:

   *A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17 and no later than the end of the Month 12 analysis visit window, that is Mtb Culture Positive (see section 4.7 for definitions of culture results) that is indistinguishable from the initial isolate (see separate sequencing plan for definitions), and this is confirmed by a second sample that is Mtb culture positive. A second confirmatory sample, on a different day (**irrespective of intervening Mtb Negative culture results**), is required, as a single positive sputum culture result in isolation will not be considered absence of bacteriological cure. If results from strain analysis are inconclusive or unavailable, it will be assumed that strains were indistinguishable.*

5. The primary efficacy analysis will be repeated reclassifying all exogenous reinfections as unfavorable.
6. The primary efficacy analysis will be repeated considering only culture inoculation results from MGIT liquid media and ignoring any culture inoculation results from solid media.
7. The primary efficacy analysis will be repeated considering only culture inoculation results from solid media and ignoring any culture inoculation results from MGIT liquid media.
8. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations excluding additionally participants for whom none of screening or baseline study visit sputum cultures are Mtb Positive (week 2 sputum cultures will not be used for determining late exclusions).
9. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations including all participants classified as randomized in error. For such patients, the classification of the outcome will follow the algorithm in section 4.1.

5.2.10 Bayesian analysis of non-inferiority

A Bayesian analysis of non-inferiority provides a more informative interpretation of the trial results by providing an estimate of the probability that the interventions regimens have efficacy not much worse than the control regimen for different thresholds of what might be considered 'not much worse'. Following methods described previously[11], Bayesian binomial regression will be used to estimate the distribution of the difference in the proportion of unfavorable outcomes between each intervention arm and the control arm, in each analysis population considering both non-informative and informative priors. These methods have also been used in secondary analyses of the STREAM Stage 1 trial (NCT02409290).

5.3 Analysis plan for secondary efficacy outcomes

5.3.1 TB disease-free survival at eighteen months after study treatment assignment

The primary efficacy endpoint for the trial is TB disease-free survival twelve months after study treatment assignment. For that primary analysis (described above), only data up to and including the 12 month visit window will be used, see section 4.1 above. Participants are followed for eighteen months from start of treatment and an important secondary efficacy outcome is therefore TB disease-free survival eighteen months after study treatment assignment.

For this analysis (TB disease-free survival eighteen months after study treatment assignment), the same definitions for the primary efficacy outcome in section 4.1 will be used with the exception that the Month 18 visit will be used in the definitions in place of the Month 12 visit. All the same analyses for the primary efficacy analysis described in section 5.2 will be repeated using this Month 18 endpoint.

5.3.2 Time to stable culture conversion

Time to stable culture conversion is defined as the time from randomization to the first of two consecutive Mtb Culture Negative results, collected on separate days without an intervening Mtb Culture Positive, and processed at the study lab of record. Participants that never achieve culture conversion will be censored at the date of collection of sputum that yielded their last negative or positive culture result.

Time to stable culture conversion will be analyzed separately for cultures from solid and liquid culture media. The Microbiologically Eligible study population will be used for these analyses. Median time to stable culture conversion will be calculated for each regimen. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.
The equality of survivor functions for time to stable culture conversion will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.
The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.
Even when Kaplan-Meier curves of time to culture conversion have been shown to diverge in the presence of an effective drug (such as bedaquiline), they tend to converge later in followup potentially violating the assumption of proportional hazards. In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.
The analyses above of time to sputum culture conversion will be repeated as follows:

1. With the alternative definition as time from randomisation to the first Mtb Negative culture from sputa processed at the study lab of record (without the need for a second negative culture to confirm).
2. Including only participants for whom 75% of more of culture results from sputum samples collected between randomization and week 12 are evaluable.

The Kaplan-Meier estimator will be used to calculate the proportion of participants with 95% confidence interval who are culture negative after 8 weeks and after 12 weeks.

5.3.3 Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection

The Microbiologically Eligible study population will be used for these analyses.
Speed of decline of MGIT days to detection will be analyzed using mixed effect non-linear regression comparing different parametric models, and taking account of the latest research and other relevant published studies. Parametric models for modelling the quantitative decline in viable bacilli used to date have included bi-exponential[12], hyperbolic tangent function[13] and surge functions[14].

5.3.4 Time to unfavorable efficacy outcome

Time to unfavorable efficacy outcome is defined as the time from randomization to the first event that results in the definition of an unfavorable efficacy outcome for that participant.
The time will be analyzed first using the primary efficacy outcome (using data from randomization to 12 months) and also using the secondary efficacy outcome at 18 months (as defined in section 5.3.1)
Participants that do not achieve culture conversion (i.e. fail to have 2 consecutive culture negative results), and have not otherwise been called unfavorable, will be called unfavorable at the date of the last visit when a culture positive result was obtained.
These analyses will be repeated for the Microbiologically Eligible, PP95 and PP75 study populations.
Participants classified as favorable or not assessable will be censored in this analysis at the date of collection of sputum that yielded their last negative culture result.

Median time to unfavorable outcome will be calculated for each regimen. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.

The equality of survivor functions will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.

6 Safety Analyses

6.1 Analysis population

All safety analyses will be conducted using the Safety Analysis Population which includes all participants randomized that took at least one dose of the allocated study regimen.

6.2 Primary safety analysis

The primary safety outcome is the proportion of participants with grade 3 or higher adverse events during the study treatment phase (defined above in section 4.6).
The difference in proportion of participants with a grade 3 or higher adverse event during study treatment phase between each of Regimens 2 and 3 compared to Regimen 1 with corresponding two-sided 95% confidence intervals will be estimated using the same methods as for the primary efficacy analysis (section 5.2.2).
It is hypothesized that the rifapentine regimens will have safety at least as good as the control regimen, but no non-inferiority margin has been pre-specified. The objective is to estimate the difference between regimens and describe the precision in this estimate (using 95% confidence intervals).
The primary safety analysis will also be repeated in the Microbiologically Eligible and Assessable analysis populations.

6.3 Secondary safety analyses

6.3.1 Treatment-related grade 3 or higher adverse events

The primary safety analysis will be repeated considering only the subset of grade 3 or higher adverse events during study treatment phase that are considered at least possibly related to study treatment.

6.3.2 Tolerability

Tolerability of the regimen is evaluated using the outcome of discontinuation of assigned treatment for a reason other than microbiological ineligibility. For this reason, participants excluded from the microbiologically eligible study population will also be excluded from this analysis.
The proportion of participants discontinuing assigned treatment will be tabulated by regimen, by week of discontinuation after date of start of treatment, and by cause of discontinuation (e.g. adverse event, withdrawal of consent, treatment failure).

6.3.3 All-cause mortality during treatment or follow-up

All-cause mortality includes all deaths from any cause during treatment or follow-up up to the end of the Month 18 visit window.
The number of participants who die during treatment and follow-up will be tabulated by regimen with listings of primary cause of death.
Median survival time will be calculated for each regimen. Participants that do not die will be censored at the date last known to be alive. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.
The equality of survivor functions will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.

6.3.4 **Serious adverse events (SAEs)**

The proportion of participants with an SAE during study treatment phase will be tabulated by regimen, MedDRA system organ class (SOC), MedDRA preferred term (PT) and severity grade. The proportion of participants with a Serious Adverse Reactions (SARs) and Suspected Unexpected SARs (SUSARs) during study treatment phase will also be tabulated by regimen and severity grade.

6.3.5 **All grade adverse events (AEs)**

The proportion of participants with any AE during study treatment phase will be tabulated by regimen, and relatedness to allocated regimen, MedDRA system organ class, MedDRA preferred term and severity grade.

6.3.6 **Laboratory safety parameters**

Laboratory safety parameters will be summarized by regimen and study visit (scaled by upper or lower limit of normal, ULN) including a summary of the highest (or lowest) measurement recorded at any visit. Summaries of drug induced liver injury (DILI) will be included using the published definition from secondary analyses of the REMoxTB trial[15] (ALT > 5x ULN and ALT > 3x ULN and total bilirubin >2 x ULN), ATS definitions and using Hy's law.
These summaries will be repeated limiting to measurements only during the study treatment phase.

6.3.7 **Acquired drug resistance**

Acquired drug resistance will be summarized by regimen, distinguishing between resistance acquired at time of treatment failure or relapse following study treatment and resistance acquired while on a non-study retreatment regimen.

7 Data summaries

7.1 Recruitment and baseline characteristics

7.1.1 Recruitment, screening, & eligibility

The number of participants screened and randomized will be tabulated by center and regimen. The number of participants who were not enrolled will be presented by center.

7.1.2 Exclusions from analysis

The number of participants excluded from each of the study populations will be tabulated by regimen and by reason for exclusion.

7.1.3 Baseline characteristics

Baseline characteristics will be tabulated by regimen. Characteristics will include sex, age, ethnicity, weight, BMI, whether underweight (BMI <18.5 kg/m^2), race, ethnicity, cavitation, radiographic severity of disease, Karnofsky score, and laboratory parameters such as, HIV status, CD4 count (if applicable), smoking status, smear and culture grading, serum albumin, hemoglobin, and time to positivity on MGIT. The baseline characteristics table will be repeated for each of study populations.

7.2 Adherence to study medications

The protocol requires at least 5 weekly doses to be provided by directly observed therapy (DOT) (i.e. participants should receive ~71% of study doses as DOT). The rest of doses are allowed to be self-administered (SAT).
Treatment adherence will be summarized by regimen first considering only DOT doses and also considering DOT and SAT doses.

7.3 Concomitant medications

Use of concomitant medications will be summarized by treatment arm considering separately 1. Non-TB and Non-antiretroviral medications, 2. Anti-retroviral medications, and 3. Additional non-study TB medications.

7.4 Retention, participant disposition, and description of follow-up

Completion of treatment and completion of scheduled follow-up will be summarized by regimen including reasons for failure to complete treatment and follow-up.

8 Other Analyses

8.1 Risk Factor Analysis

Univariate and multivariate analyses will be carried out to assess the association of unfavorable outcomes and clinical and demographic variables using Cox regression (time to event endpoints) and logistic regression (binary endpoints). Variables included as potential risk factors include baseline data in addition to on-treatment data such as time to culture conversion and weight gain. The most parsimonious model that contains significant variables with $p<0.05$ will be used to identify risk factors. The interactions between risk factors and treatment regimens will be examined to evaluate variations of different risk factors among treatment regimens.

8.2 Efavirenz Pharmacokinetics Analysis

Efavirenz pharmacokinetics will be evaluated in the first 31 and then a total of 90 participants from each of two groups of participants randomized to treatment regimen 2 or 3 of the study and receiving efavirenz based ART. The analysis plan for this is described in a separate statistical analysis plan (TBTC Study 31 Efavirenz Pharmacokinetic Statistical Analysis Plan).

9 Interim monitoring by the Data and Safety Monitoring Board (DSMB)

Interim analyses will be provided to the DSMB for monitoring the efficacy and safety and for the DSMB to review other matters occurring during the trial. Based on the interim results, the DSMB may recommend early closure of any experimental arm or the trial if the interim evidence is sufficiently strong that one of the trial interventions is clearly indicated or clearly contraindicated because of a net difference in efficacy, safety, or tolerability.

If the evidence provides proof beyond reasonable doubt that there is a clear and meaningful benefit or harm of a given regimen, the DSMB may recommend termination of one of the treatment arms or termination of the study.

9.1 Monitoring for efficacy

Two interim analyses for the primary efficacy outcome are planned. To provide guidance to continue, adjust or stop the trial, the two interim analyses will be carried out after 40% and 60% of participants, respectively, reach 12 months post-randomization, and allowing for up to an additional four months for reporting of all culture results. The primary efficacy outcome will be conducted according to the statistical analysis plan described in section 5.2 with the analysis populations described therein.

Specifically, the primary analysis of the primary outcome will be conducted for both MITT and per protocol analysis populations. Non-inferiority hypotheses will not be evaluated, only superiority hypothesis – whether either intervention is superior to control. It is generally not recommended to stop a trial early for early evidence of non-inferiority as obtaining more information is usually beneficial, unless superiority has been demonstrated[16].

There will be no ordering of hypotheses in the interim analyses.

The Haybittle-Peto stopping boundaries are to be used for the interim analyses. Following the Haybittle-Peto rule in monitoring the efficacy, the statistical criterion for the differences between arms is set at the 0.1% level of statistical significance in each of the two interim analyses. If any arm is shown to have superior efficacy to the control arm at the 0.1% level of statistical significance (i.e. using 99.9% confidence intervals), the DSMB should consider whether it would be appropriate to terminate some arms or the whole trial, taking into account other efficacy and safety analyses. The overall type I error rate for testing non-inferiority and superiority is unaffected by the addition of these interim analyses using the Haybittle-Peto stopping boundaries due to the high level of evidence required (99.9% confidence intervals)[17].

9.2 Monitoring for safety

The DSMB will convene approximately annually, or more often as needed, to review safety data. The safety monitoring in the interim analyses will focus on the comparison of the proportion of participants with adverse events in each treatment arm, with a particular focus on the primary safety endpoint. Concerns about the extent and type of adverse events observed may lead to early termination of the trial when the DSMB judges that the potential benefits of the new regimens are unlikely to outweigh the risks. In other cases, the DSMB may recommend measures short of termination that may reduce the risk of adverse events. For example, modifying the inclusion criteria if the risks are concentrated in a particular subgroup or instituting screening procedures that could identify those at increased risk of a particular adverse event.

9.3 **Unblinded statistician**

If the primary trial unblinded statistician takes on the responsibility for the interim analysis and reporting to the DSMB, this statistician will not participate in any discussions about further changes to the trial protocol, and statistical input from a blinded statistician will be sought as necessary.

10 Version history

Version 1.0	12 December 2018
Version 1.1	16 April 2019
Version 2.0	09 July 2019

10.1 List of changes from version 1.0 to version 2.0

Added expanded justification for margin of non-inferiority in section 1.4.
Added section 5.2.4 'Comparison of intervention arms' to describe exploratory comparison of the two intervention regimens.
Added in section 9.1 further clarification of procedures at interim analyses, namely superiority hypothesis testing for superiority only not non-inferiority and clarifying that overall type I error is not affected by Haybittle-Peto stopping rules.
Added section 9.3. Unblinded study statistician describing the role of the unblinded trial statistician and their absence from any protocol design changes.

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Addendum to the Statistical Analysis Plan for TBTC Study 31 / ACTG A5349

Version: 1.0

22 Jul 2020

Approved: Patrick Phillips, Trial Statistician

DocuSigned by:
Patrick Phillips
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7/22/2020

1 Purpose

This document is an addendum to the Statistical Analysis Plan (SAP) of the TBTC Study 31 / ACTG A5349, is version 2.0, 9 July 2019.

The purpose of this document is two-fold:

1. to provide further clarification on specific definitions in the SAP where the wording was unclear, and
2. to describe further sensitivity analyses of the primary efficacy endpoint.

This document does not propose any modifications or changes to the analysis plan for the trial beyond wording clarification and specification of further sensitivity analyses.

2 Clarification of Analysis Plan Definitions

2.1 Pregnancy during assigned study treatment

Section 4.1.3 of the SAP describes criteria for classification of study participants as having a Not Assessable outcome. The second item in the list specifies that 'Women who become pregnant during assigned study treatment' are classified as Not Assessable. This item is intended to refer specifically to women who become pregnant and for whom treatment is changed due to pregnancy. This item therefore does not apply to women who become pregnant during assigned study treatment where treatment is not changed due to pregnancy.

3 Additional Sensitivity Analyses

Section 5.2.9 of the SAP describes nine additional sensitivity analyses. The following five sensitivity analyses will also be conducted:

10. The primary efficacy analysis will be repeated relaxing the criteria for evaluable cultures by removing the inoculation result classification of 'Unevaluable' so that inoculations are reclassified as positive, negative, contaminated, or missing.
11. The primary efficacy analysis will be repeated reclassifying participants classified as Not Assessable because they did not attend a visit within the Month 12 analysis visit window based on the immediate next data available for the participant after the Month 12 analysis visit window. If the patient is culture negative at the next visit after the Month 12 visit window, they will be classified in this analysis as favorable, if they are culture positive at the next visit at the Month 12 visit window, they will be classified in this analysis as unfavorable. This sensitivity analysis will be interpreted with caution as, at the time of the 12-month primary analysis when all participants will not have completed 18 months of follow-up, it will include a mix of month 15 and month 18 data.
12. The primary efficacy analysis will be repeated reclassifying patients that have two positive cultures but do not have subsequent restart of treatment as favorable rather than unfavorable.
13. The primary efficacy analysis will be repeated with modified analysis visit windows for visits after Month 9 according to the following table:

Visit	Target date (days from date of first dose of treatment)	SAP v2.0 Analysis window for primary analysis	Analysis window for sensitivity analysis
Month 9	270	263-352	263-**345**
Month 12	360	353-442	**346**-442
Month 15	450	443-523	443-**509**
Month 18	540	533-no upper bound (an upper bound of 570 will be used for reporting safety analyses)	**510**-no upper bound (an upper bound of 570 will be used for reporting safety analyses)

    The analysis visit windows for the primary Month 12 visit and the end of follow-up Month 18 visit extend to 14 and 30 days prior to the target date of visit respectively (rather than 7 days for other study visits) since these are critical visits for the primary and end of follow-up efficacy analyses.
14. The primary efficacy analysis will be repeated reclassifying as Cure (Favorable) those participants who have not achieved culture conversion prior to Month 12, but are otherwise seen during the Month 12 analysis visit window and are clinically without symptoms/signs or ongoing active TB and fulfill all the other criteria under the second item under section 4.1.2.

Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis:

A randomized, open-label, controlled phase 3 clinical trial

Consortium Identifiers:

Tuberculosis Trials Consortium Study 31

AIDS Clinical Trials Group A5349

ClinicalTrials.gov Identifier: NCT02410772

Whole Genome Sequencing

Analysis Plan

(WGSAP)

Version Number: 1.0

7 July 2020

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Approval: Jamie Posey, TB Research Lab Team Lead

Patrick Phillips, Trial Statistician

General Information

This document describes and substantiates principles and methods used for the analysis of whole-genome sequencing (WGS) data from trial TBTC S31/ACTG A5349. This document is designed to support the trial protocol and the trial statistical analysis plan (SAP).

Version 1.0 of this Whole Genome Sequencing Analysis Plan (WGSAP) was signed off prior to the inclusion of WGS data to the database extract for the primary efficacy analysis.

1. Introduction

1.1 Purpose

Culture results that occur after randomization are important for classification of the primary efficacy outcomes for the primary analysis. Furthermore, whether the strain of *M. tuberculosis* complex (MTBC) is the same or different from the baseline isolate, also affects classification. The trial statistical analysis plan (SAP) describes in detail how the primary efficacy outcome is defined but does not include information on rules for when two strains can be considered indistinguishable versus not indistinguishable.

2. Methods and Procedures

1.2 Definitions and classification for reporting WGS

Indistinguishable isolates: Difference of <10 single-nucleotide polymorphism (SNPs) between paired isolates.

Not indistinguishable isolates: Difference of ≥10 single-nucleotide polymorphism (SNPs) between paired isolates.

Inconclusive: Sequence was not obtained or did not meet quality threshold from at least one isolate of the isolates pair.

Unavailable isolates: At least one of the isolates from the pair was not shipped from the site or was not received at the CDC Laboratory.

1.3 Handling of Mixed infections

The following approach will be applied if mixed infection is detected using current methods.

- WGS of MTBC culture obtained at or after week 17 suggests a mix of the baseline isolate and a different isolate: will be classified as **Indistinguishable**.

- Baseline MTBC culture has mix of two isolates. WGS of MTBC culture obtained at or after week 17 suggests a mix of the baseline isolates (or one of the baseline isolates) and a different isolate: will be classified as **Indistinguishable**.

- WGS of MTBC culture obtained at or after week 17 suggests a mix of two different strains as compared to baseline isolate: will be classified as a **Not indistinguishable**.

- WGS of MTBC culture obtained at or after week 17 suggests a mixed infection, but we are unable to determine in the baseline strain or close variant (<10 SNPs) **Inconclusive**.

Cut-off for 10 SNPs is based on CDC's experience using wgSNP analysis from routine WGS for all US strains for transmission analysis and used in the standard WGS molecular epidemiology done as part of domestic TB surveillance.

1.4 Selection of isolates to be sequenced

The following isolates will be sequenced:

- The first isolate available per participant (or isolate linked in time to TB resurgence) that is obtained at week 17 or after post-randomization
- A corresponding baseline isolate to the participant above
- Further isolates if study team requests WGS to provide help differentiate relapse vs. reinfection, decided on a case by case basis

Exclusion criteria:

- Visibly contaminated isolates will not be sequenced
- Isolates that did not produce enough growth on subculture will not be sequenced
- Isolates for which sequences obtained contain <90% MTB DNA will be excluded from analysis
- Isolates for which sequences obtained include <50x coverage on repeated extraction

1.5 Sample shipment and isolate banking

Samples are shipped from laboratories serving all sites and are received and processed by the Laboratory Branch at CDC (LB-CDC). LB-CDC will request reshipment of grossly contaminated samples and will attempt decontamination and subculture in cases where TB colonies are isolated. The use and banking of the isolates from Study 31/A5349 is guided by material transfer agreements (MTAs) with each of the sites and may have differing clauses for storage and sharing.

1.6 DNA extraction and WGS

Chromosomal DNA will be extracted from 200 uL of a frozen stock using the Zymo Quick DNA Fungal/Bacterial kit and quantified using the Qubit 3.0 high sensitivity assay. Sequencing libraries will be generated using the Nextera XT library prep kit following standard protocols. The libraries will be sequenced (2 x 250bp reads) on the Miseq platform using Miseq Reagent Kit v3 600 cycle chemistry. Samples with a sequence read set Q30 frequency <80% will be repeated.

1.7 WGS data processing and analysis

The presence of DNA not from MTBC organisms will be detected with the microbial classification engine Centrifuge. Samples containing less than 90% MTBC will be excluded from further analysis.

Reference guided assemblies will be created using Bionumerics 7.6 Reference Mapper v 1.2.3 using *M. tuberculosis* strain H37Rv (NC00962.3) as the reference with the following settings for base calling: Minimum total coverage=3, Minimum forward coverage=1, Minimum reverse coverage=1, Single base threshold=0.75, double base threshold=0.85, triple base threshold=0.95, gap threshold=0.5. Bowtie based algorithm described in reference. Samples with an average genome coverage less than 50 will be resequenced.

Reference guided assemblies for baseline sample and for the first sample available sample collected at or after 17 weeks (or isolate linked in time to TB resurgence) will be compared using Bionumerics V7.6 SNP analysis filters. For a SNP to be retained in the comparison, the base in both samples must have a total coverage of 5 reads, must not contain ambiguous bases, must not contain unreliable bases, must not contain gaps, and must not be within 12 basepairs of another SNP. SNPs that are non-informative (identical in both samples) were also excluded. The number of high-quality SNPs between the two samples will be recorded as the SNP distance.

Paired isolates with <10 SNPs will be considered **indistinguishable** and not further analyzed. Paired isolates with a SNP distance >25 SNPs will be considered **not indistinguishable**. In cases when SNP distance is between 10 and 25 SNPs a manual curation will be used to look at every SNP location and verify that all the calls have appropriate coverage and sequence meets quality threshold. The purpose of manual curation to ascertain that difference is ≥10 SNPs (or not) and to confirm SNP quality. If the difference is ≥10 true SNPs isolates will be considered **not indistinguishable**.

To rule out the presence of a mixed infection in a single sample, variant call files will be analyzed for the presence of multiple phylogenetic SNPs. The detection of greater than one phylogenetic SNP will suggest mixed infections. To rule out the presence of a mixed infection in either one of the paired samples where one sample contains both the strain in the first sample as well as a second strain, variant call files will be examined. SNPs fixed in one sample (identified in >90% of the reads) but mixed in the corresponding sample (present in 25-75% of the reads) are identified and counted. Difference greater than 25 SNPs will suggest that the sample contains two strains.

1.8 Incorporation of WGS data into Study S31/A5349 database

A spreadsheet containing a line list per participant with SNPs difference between the paired baseline isolate and positive isolate(s) from week 17 or after will be shared with Clinical Research Branch (CRB) and merged with the main TBTC2 study database.

1.9 Software used

Bionumerics V7.6 (Applied Maths, Belgium)

Centrifuge v1.0.3 (https://github.com/infphilo/centrifuge/releases)

1.10 References

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5.1 Contact info

Lab Branch: Jamie Posey (hzp9@cdc.gov)

Clinical Research Branch: Jessica Ricaldi (mpi7@cdc.gov)

6 Version history

Version 1.0	7 July 2020

Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis:
A randomized, open-label, controlled phase 3 clinical trial

Consortium Identifiers:
Tuberculosis Trials Consortium Study 31
AIDS Clinical Trials Group A5349

ClinicalTrials.gov Identifier: NCT02410772

Statistical Analysis Plan

Version Number: 1.0
12 December 2018

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Approval:

Patrick Phillips, Trial Statistician

PPhillips 2/JAN/2019

Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis:
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Consortium Identifiers:
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Statistical Analysis Plan

Version Number: 1.0
12 December 2018

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Approval:

Patrick Phillips, Trial Statistician

General Information

This document describes and substantiates the statistical principles and methods used for the analysis of data from TBTC S31/A5349. This document is designed to support the trial protocol. This Statistical Analysis Plan (SAP) supersedes previous versions of the SAP. Every care was taken in the drafting of this SAP, but corrections or amendments may be necessary.

Version 1.0 of the SAP will be signed off prior to the database extract for the first interim efficacy analysis.

Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR, and applicable site-specific regulatory requirements.

Contents

1 Introduction

1.1 Protocol Summary

Title: Rifapentine-containing treatment shortening regimens for pulmonary tuberculosis: a randomized, open-label, controlled, phase 3 clinical trial

Hypotheses:	A) Seventeen (17) week rifapentine-based regimen In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine (P), isoniazid (H), pyrazinamide (Z) and ethambutol (E) followed by nine weeks of rifapentine plus isoniazid, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin (R), isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout. B) Seventeen (17) week rifapentine- plus moxifloxacin-containing regimen In previously untreated individuals with active drug-susceptible pulmonary tuberculosis treated with eight weeks of rifapentine, isoniazid, pyrazinamide and moxifloxacin (M), followed by nine weeks of rifapentine, isoniazid, and moxifloxacin, all given daily throughout, the proportion of participants who experience absence of cure (unfavorable outcome) will not be inferior to that observed in participants who are treated with a standard regimen (eight weeks of rifampin, isoniazid, pyrazinamide and ethambutol followed by eighteen weeks of rifampin plus isoniazid), all given daily throughout.
Phase:	3
Design:	This will be an international, multicenter, randomized, controlled, open-label, 3-arm, phase 3 non-inferiority trial.
Population:	Participants with newly diagnosed, previously untreated pulmonary tuberculosis.
Number of Sites:	Multiple international sites, primarily sites of the Tuberculosis Trials Consortium and the AIDS Clinical Trials Group.
Study Duration:	Duration per participant is approximately 18 months.

Description of Agent or Intervention: After written informed consent, participants will be randomly assigned to receive one of the following oral regimens:

Regimen 1 (control regimen): 2RHZE/4RH

- Eight weeks of daily treatment with rifampin, isoniazid, pyrazinamide, and ethambutol, followed by
- Eighteen weeks of daily treatment with rifampin and isoniazid

Regimen 2 (investigational regimen): 2PHZE/2PH

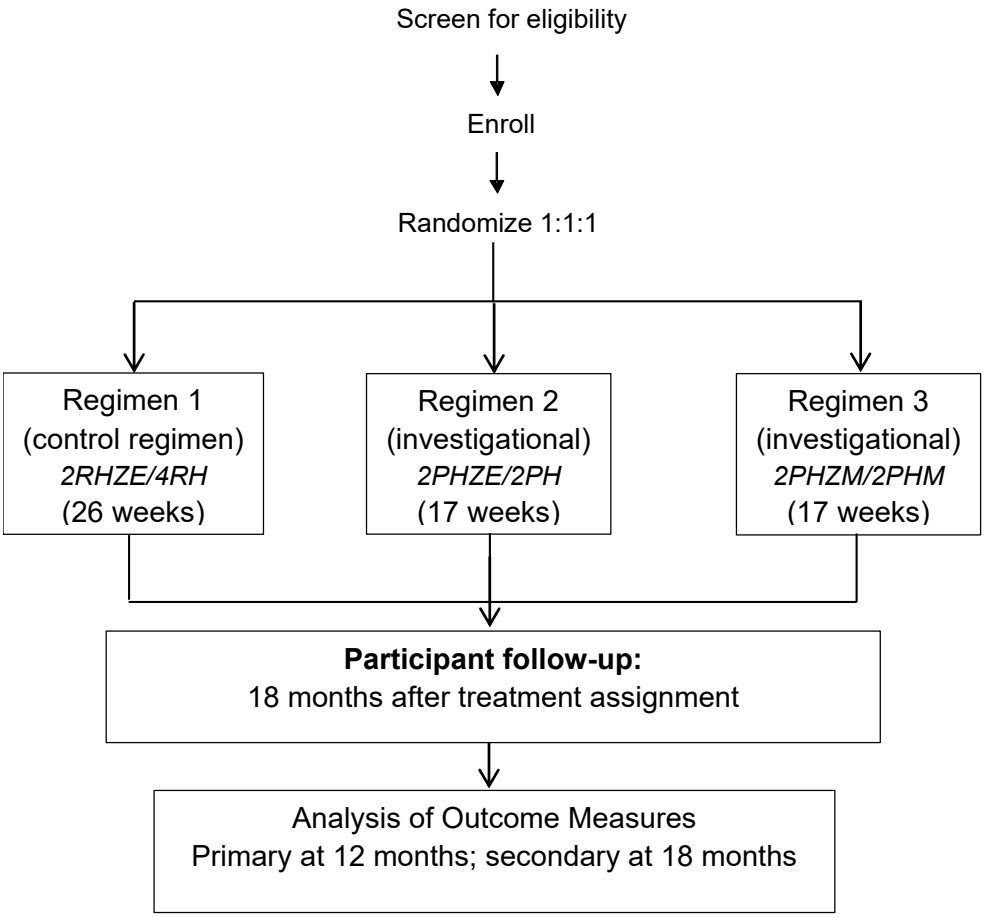
- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and ethambutol, followed by
- Nine weeks of daily treatment with rifapentine and isoniazid

Regimen 3 (investigational regimen): 2PHZM/2PHM

- Eight weeks of daily treatment with rifapentine, isoniazid, pyrazinamide, and moxifloxacin, followed by
- Nine weeks of daily treatment with rifapentine, isoniazid, and moxifloxacin

1.2 Schematic of study design

Figure 1 Schematic of study design



1.3 Sample size considerations

The primary objective of the trial is to evaluate whether rifapentine containing regimens can produce outcomes at least as favorable as standard therapy, but with a shorter treatment course. Therefore, the trial is structured as a non-inferiority study.

Key assumptions:

- Primary endpoint rate: 15% absence of cure (unfavorable) in the standard regimen arm (Microbiologically Eligible population). This rate is based on observed results for the control arm (MITT analysis group) in two recently completed phase 3 clinical trials (27/161 [14%] in the Rifaquin trial[1] and 100/743 [13.5%] at 18 months post randomization and 114/679 [16.8%] at 24 months after the end of treatment in the Oflotub trial[2].)
- Margin to define inferiority: 6.6% ($\delta = 0.066$)
- 95% confidence (type 1 error, $\alpha = 0.05$). The sequential testing of regimen 3 and regimen 2 protects the type 1 error rate, as follows: If the statistical test for regimen 3 fails at 95% confidence, then conclude that both experimental regimens are not noninferior. If and only if regimen 3 is noninferior, then proceed to test regimen 2 at 95% confidence. A type 1 error occurs if either regimen is incorrectly deemed noninferior; the sequential approach limits the probability of this error to 5% overall.
- Power: 80% (type 2 error, $\beta = 0.20$) for primary analysis among Microbiologically Eligible subgroup, with power recalculated for the restriction to Assessable subgroup (see below)
- Proportion of enrolled participants who would be found to be late exclusions due to microbiological ineligibility – 12% (based on observed results in recent TBTC phase 2 studies)
- Proportion of enrolled participants who would be found to be 'not assessable': 12% (based on observed results in the Rifaquin trial[1])

With 816 per arm, we expect 612 assessable. With the expected 15% unfavorable outcomes among those who are assessable, then with the same noninferiority margin and type 1 error rate, we have 90% power to test the primary hypotheses among the Assessable subgroup.

1.4 Margin of non-inferiority

The 6.6% margin to define inferiority (6.6%) takes into consideration the following issues:

(1) the rates in historical trials of inpatient TB treatment for 6-month and 4-month regimens conducted by the British Medical Research Council support a difference in relapse up to 6% (East African/British Medical Research Council 1976, 1977, 1981; East and Central Africa/British Medical Research Council 1986; Singapore Tuberculosis Service/British Medical Research Council 1986; Nunn and Crook 2013);

(2) recent trials in contemporary outpatient populations suggest a higher baseline proportion (15%) of unfavorable outcomes likely to be observed based on phase 3 trials and definitions;

(3) the investigators in this trial and others perceive that the benefits of reducing treatment duration to 3 or 4 months would have advantages not outweighed by a possible increase in the relapse rate of up to 6%; and

(4) the 6.6% margin does not imply that the experimental regimen may result in as much as 6.6% more unfavorable outcomes, but rather, for a fixed design, the maximum difference consistent with a non-inferior conclusion decreases as the proportion of unfavorable outcomes in the control arm increases.

A 6% margin of non-inferiority trials has been used in other recent trials of single-drug substitution treatment shortening trials (e.g. REMoxTB). The justification of this margin is published in the online supplements with these papers (Gillespie et al, 2014 NEJM). We have attached the justification from that study as an attachment to support a 6% margin.

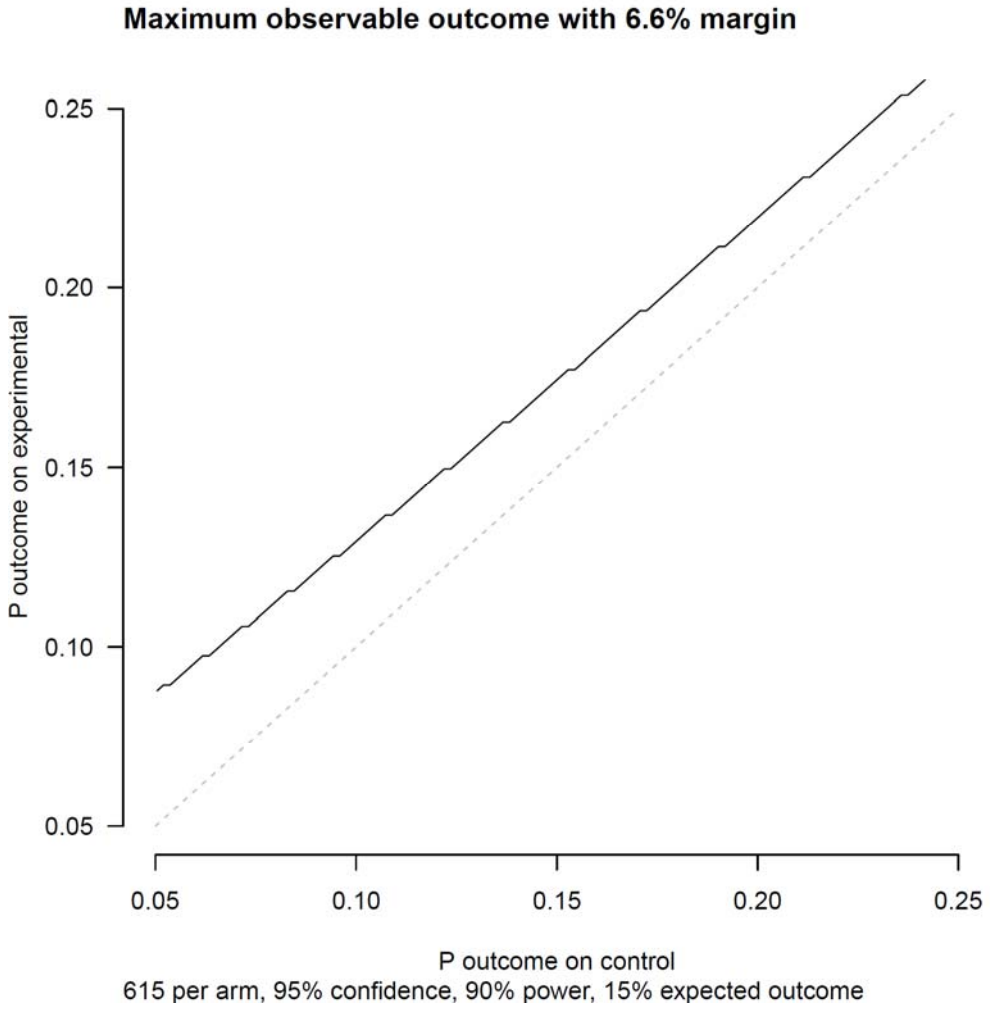
We believe an extension from 6% to 6.6% is justified for the following reasons:

1) The justification for a 4.8% margin in the FDA Guidance for Industry for Pulmonary Tuberculosis Trials is based on previous trials under 'per protocol' type analyses with many post-randomization exclusions, in a largely in-patient population; we observe larger proportions of unfavorable outcomes today than was seen in these relapse-only analyses from previous trials. We feel this provides a justification for a larger margin than 4.8%, and also the 6% that was justified for the REMoxTB trial. Recent trials in contemporary outpatient populations suggest a higher proportion (16% in REMoxTB MITT) of unfavorable outcomes, even than that anticipated in the REMoxTB trial sample size calculations (10%).

   Furthermore, the rationale for a 4.8% margin is based on the situation where a single drug that has an unknown contribution to the regimen is replaced by a new drug (the replacement of ethambutol, for example). In our study, rifampicin is replaced by rifapentine (in addition to the substitution of moxifloxacin for ethambutol in one arm). It is known that rifampicin is the most important drug in the current regimen. It might therefore be appropriate to consider not just the removal of the final two months of therapy (following the argument in lines 829-832 in the FDA Guidance) to estimate M_1, but also the consider the removal of rifampicin from the regimen. This would require consideration of a comparison of six months of HRZE (2HRZE/4HR) with four months of HZE (2HZE/2H) when estimating M_1. We are not aware of any trials that evaluated a 4-month regimen without rifampicin, so providing a comprehensive rationale similar to that which underpins the 4.8% would be challenging but would lead to a larger M_1 and therefore support a margin of non-inferiority larger than 4.8%.

2) Considering the clinical argument (from FDA Guidance and Nunn, Phillips, Gillespie 2008) we, and in broader consultation within our two large publicly-funded international consortia of TB stakeholders (CDC TB Trials Consortium and NIH AIDS Clinical Trials Group), consider the benefits of a 4-month rifapentine-based regimen justify the margin of 6.6%. Our consortia consider 600 patients per arm sufficiently large to provide adequate precision on the difference in efficacy between the regimens to determine whether an intervention regimen might be considered not inferior to the control regimen.

   The following graph was used to describe the maximum observable difference (solid line) from in the point estimate from the line of equality (plotted as dashed line) with a 6.6% margin under the stated assumptions.



For these reasons it is our perspective that a margin of 6.6% is justified.

2 Objectives

2.1 Primary Objectives

- To evaluate the efficacy of a rifapentine-containing regimen to determine whether the single substitution of rifapentine for rifampin makes it possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis
- To evaluate the efficacy of a rifapentine-containing regimen that in addition substitutes moxifloxacin for ethambutol and continues moxifloxacin during the continuation phase, to determine whether it is possible to reduce to seventeen weeks the duration of treatment for drug-susceptible pulmonary tuberculosis

2.2 Secondary Objectives

- To evaluate the safety of the investigational regimens
- To evaluate the tolerability of the investigational regimens
- To collect and assess biospecimens from consenting participants for the purpose of research on discovery and validation of TB biomarkers
- To determine the correlation of mycobacterial and clinical markers with time to culture conversion, treatment failure, and relapse.
- To conduct a pharmacokinetic/pharmacodynamic (PK/PD) study of the test drugs. The main objectives of the PK/PD study are to characterize study drug PK parameters and to determine relationships between treatment outcomes and PK parameters.
- To evaluate the pharmacokinetics of efavirenz-based antiretroviral treatment among participants with TB/HIV co-infection taking efavirenz-based combination antiretroviral therapy and TB treatment with rifapentine.

3 Endpoints

3.1 Primary Endpoints

- Efficacy: TB disease-free survival at twelve months after study treatment assignment.
- Safety: Proportion of participants with grade 3 or higher adverse events during study drug treatment

3.2 Secondary Endpoints

- TB disease-free survival at eighteen months after study treatment assignment
- Time to stable sputum culture conversion (solid and liquid media considered separately)
- Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection
- Proportion of participants who are culture negative at completion of eight weeks of treatment (solid and liquid media considered separately)
- Sensitivity analyses assuming all participants classified as ‘not assessable’ have a favorable outcome
- Discontinuation of assigned treatment for a reason other than microbiological ineligibility
- Estimated steady state efavirenz PK parameters including mid-dosing interval concentration

4 Study definitions

4.1 Definition of primary outcome

Each participant will be classified into one of the following three outcome categories:

1. Absence of Cure (Unfavorable Outcome)
2. Cure (Favorable Outcome), or
3. Not assessable.

The primary outcome is defined as twelve months after study treatment assignment. Actual visit dates, rather than scheduled visit names (e.g. Week 26, or Month 9), will be used for all analyses. See section 4.5 for visit windows that define the time periods. In particular, Month 12 includes data from visits up to 442 days from treatment initiation.

Only data up to the end of the Month 12 analysis visit window will be included in the primary analysis of the primary efficacy outcome.

4.1.1 Absence of Cure (Unfavorable)

A participant will be classified as having an unfavorable outcome if any one of the following conditions is met:

1. A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17 and no later than the end of the Month 12 analysis visit window, that is Mtb Culture Positive (see section 4.7 for definitions of culture results) that is indistinguishable from the initial isolate (see separate sequencing plan for definitions), and this is confirmed by a second sample that is Mtb culture positive. A second confirmatory sample, on a different day without an intervening Mtb Negative culture result, is required, as a single positive sputum culture result in isolation will not be considered absence of bacteriological cure. If results from strain analysis are inconclusive or unavailable, it will be assumed that strains were indistinguishable.
2. Participants who die from any cause during study treatment ('study treatment phase' is defined in section 4.6), except from violent or accidental cause (e.g. road traffic accident). Suicide during study treatment will be classified as an unfavorable outcome.
3. Participants who are withdrawn from follow-up or lost to follow-up prior to the scheduled end of treatment of study treatment, except for pregnancies and violent or accidental death that are instead classified as having a Not Assessable outcome (see section 4.1.3).
4. Participants who had an Mtb Positive culture result when last seen during or prior to the Month 12 analysis visit window, whether confirmed by a second sample or not, unless determined to have been re-infected.
5. Participants receiving any one or more of the following, except when given for failure or recurrence subsequently shown to be a reinfection with a strain of M. tuberculosis, different from that or those identified at study entry through genotyping methods):
    a) Extension of treatment beyond that permitted by the protocol; excepting
        a. Temporary drug re-challenge;
        b. Over-treatment with drugs from assigned study kits;
        c. Twenty-one days or fewer of non-study anti-TB medications given for treatment of active TB; or
        d. Secondary isoniazid preventative therapy in HIV infected participants.
    b) Re-start of treatment for active TB;
    c) Change in treatment (including frequency or dosage) for any reason except re-infection, pregnancy, or temporary drug challenge.
6. Participants who die during the follow-up phase (as defined in section 4.6) where the cause of death is considered related to tuberculosis.

4.1.2 Cure (Favorable)

A participant will be classified as having a favorable outcome if any one of the following conditions is met and an unfavorable outcome has not occurred:

1. Participants whose last culture result during the Month 12 analysis visit window is Mtb Negative (See Section 4.7).
2. Participants who are seen during the Month 12 analysis visit window and are clinically without symptoms/signs of ongoing active TB (indicated by absence of initiation of possible poor treatment response evaluation or PPTR that does not indicate presence of symptoms/signs of ongoing active TB), and have achieved culture conversion prior to Month 12, and
    1. Are unable to produce a sputum specimen at any point during the Month 12 analysis visit window; or
    2. Produce a sputum specimen that is contaminated or unevaluable without evidence of *M. tuberculosis,* and no sputum specimens yield positive or negative culture results during the Month 12 analysis visit window.

4.1.3 Not Assessable

A participant will be classified as having a Not Assessable outcome if any one of the following conditions is met and an unfavorable outcome has not occurred:

1. Participants not otherwise classified as unfavorable, but do not attend a visit within the Month 12 analysis visit window, and their last culture result is negative for *M. tuberculosis.*
2. Women who become pregnant during assigned study treatment (see section 4.6 for definition of study treatment phase).
3. Participants who die during the follow-up phase (as defined in section 4.6) of any cause that is not considered related to tuberculosis.
4. Participants who die from a violent (e.g. homicide) or accidental (e.g. road traffic) cause during their assigned study treatment (see section 4.6 for definition of study treatment phase). As above, suicide will be considered an unfavorable outcome.
5. Participants who are:
    a) Retreated, or have treatment changed or extended; and
    b) Demonstrated to be re-infected with a strain of M. tuberculosis, different from that or those identified at study entry through genotyping methods.

A participant classified as having a Not Assessable outcome will be excluded from the Assessable and Adherent Per-Protocol analyses, but considered as Unfavorable for other analyses.

4.2 Participants Randomized in Error

Participants who were randomized in error are those who were found to not meet eligibility criteria after enrollment, other than criteria in Section 4.3.

Determination of whether eligibility criteria was violated and subsequent classification as ‘randomized in error’ will be based only on data that was collected prior to randomization. All participants who are found to be in violation of any eligibility criteria (other than the criteria in Section 4.3 relating to microbiology) will be classified as randomized in error, irrespective of whether the participant was withdrawn from treatment or not.

4.3 Exclusion criteria after enrollment (‘Late Exclusion’)

Microbiological confirmation of drug-susceptible tuberculosis is not expected always to be available at the time of enrollment. Enrolled individuals who are subsequently determined to

meet either of the following criteria will be classified as ‘late exclusions’ and study treatment will be discontinued:

1. None of screening, baseline, and Week 2 study visit sputum cultures are Mtb Positive
2. *M. tuberculosis* cultured or detected through molecular assays (Cepheid Xpert MTB/RIF or Hain MTBDR*plus* assays) from any sputum obtained at screening, baseline, or week two study visit is determined subsequently to be resistant to one or more of isoniazid, rifampin, or fluoroquinolones.

4.4 Adequate treatment

Only participants having completed an adequate number of study doses will be included in the Per Protocol (PP) analysis populations. Two PP analysis populations are defined. PP75 excludes participants who have received less than approximately 75% of study doses (see Table below for exact doses required) using the definitions consistent with previous phase III TB trials, in particular the REMoxTB trial[3] and in the original trials which determined the effectiveness of the control 6 month isoniazid-rifampin regimen[4]. The TB-REFLECT analyses[5] have shown that even participants with less than 95% adherence have poorer outcomes than those with perfect adherence, and consecutive missed doses is associated with poorer outcomes than occasional missed doses[6]. For these reasons, the PP95 analysis population excludes participants who have received less than approximately 95% of study doses (see Table 1 below for exact doses required). PP95 will be the primary per protocol analysis population with PP75 being supportive.

Table 1. Two definitions of adequate treatment

	75% Adherence (PP75) Approximately 75% of doses within 125% of the intended duration.		95% Adherence (PP95) Approximately 95% of doses within 125% of the intended duration.	
	Doses	Days	Doses	Days since treatment initiation
Regimen 1	At least 42 intensive phase doses	No more than 70 days since treatment initiation	At least 54 intensive phase doses	No more than 70 days since treatment initiation
	At least 84 continuation phase doses	No more than 168 days since completing intensive phase	At least 120 continuation phase doses	No more than 168 days since completing intensive phase
	No more than 42 doses missed		No more than 5 consecutive DOT doses missed	
Regimens 2 or 3	At least 42 intensive phase doses	No more than 70 days since treatment initiation	At least 54 intensive phase doses	No more than 70 days since treatment initiation
	At least 42 continuation phase doses	No more than 84 days since completing intensive phase	At least 60 continuation phase doses	No more than 84 days since completing intensive phase
	No more than 28 doses missed		No more than 5 consecutive DOT doses missed	

4.5 Visit windows

Participants are assessed at screening, baseline (Week 0), Week 2, Week 4, Week 8, Week 12, Week 17, Week 22, Week 26, Month 9 and 3-monthly to Month 18 with visit dates scheduled from the date of treatment initiation.

For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of first dose of study medication. The only exception is the visit window for Baseline which is from date of screening consent to date of randomization (inclusive). Any visits after randomization up to 24 days after treatment initiation are included in the Week 2 visit window.

When referring to a visit in this analysis plan (e.g. 'Week 17'), this implies within the defined visit window as specified below in Table 2.

Table 2. Analysis visit windows

Visit	Target date (days from date of first dose of treatment)	Analysis window
Baseline (including screening period)	Date of randomization	Date of screening consent – Date of randomization
Week 2	14	Day after randomization-24
Week 4	28	25-52
Week 8	56	53-80
Week 12	84	81-115
Week 17	119	116-150
Week 22	154	151-178
Week 26	182	179-262
Month 9	270	263-352
Month 12	360	353-442
Month 15	450	443-523
Month 18	540	533-no upper bound (an upper bound of 570 will be used for reporting safety analyses)

Any visit, scheduled or unscheduled (including possible poor treatment response or post early termination visits), that falls into the analysis window will be assigned to that visit for the purpose of analysis. If two visits fall within the same interval, the one closest to the target date will be used for analyses by visit, or the highest value, depending on the analysis, so that there is only one unique visit for each participant and analysis time-point. However, all critical endpoint determining data will be used (e.g. culture results).

4.6 Treatment and follow-up phases

For the purposes of analysis, the screening, treatment and follow-up phases are defined as follows:

- **Pre-treatment phase**
  - Start: date informed consent signed.
  - End: day before start of study treatment.

- **Study treatment phase**
  - Start: date of start of study treatment.
  - End: date of last dose of allocated treatment regimen, plus 14 days.

- **Follow-up phase**
  - Start: the day after the end of the study treatment phase.
  - End: date of the last participant contact (scheduled or unscheduled, or other contact e.g. phone call).

4.7 Microbiologic classification

4.7.1 Inoculation results

Each sputum specimen is inoculated onto solid and liquid media. The result of each inoculation is classified as Mtb Positive, Mtb Negative, Contaminated, or Unevaluable (Table 3). It is expected that one sputum specimen will produce two inoculation results, one for each media type. Inoculation results reported as Mtb alone, Mtb with other mycobacteria (e.g., NTM), or Mtb with contamination will be classified as Mtb Positive. Inoculations reported as no growth or other mycobacteria without identification of Mtb will be classified as Mtb Negative. Inoculations reported as contaminated without identification of Mtb will be classified as Contaminated.

Table 3. Classification of culture results per inoculum

Inoculation Result Classification	**Definition**
Mtb Positive	**Definitive identification of Mtb with or without the presence of contaminants or other mycobacteria.**
Mtb Negative	**No Mtb or contaminants detected at least 42 days after inoculation. Growth of other mycobacteria (NTM) in the absence of Mtb or contaminants.**
Contaminated	**Growth of contaminants with or without other mycobacteria, but with no identification of Mtb.**
Unevaluable	**Includes any of the following:** • Sputum processed at non-lab of record. • Media inoculated more than 4 days after sputum received in the lab. • Missing culture result. • Mtb identification was presumptive with no definitive identification. • Result of no growth determined fewer than 42 days after inoculation.

4.7.2 Culture results

The primary analysis will be conducted using results from inoculation onto solid and liquid media. For each sputum specimen, results from solid and liquid media inoculations will be combined into a single Culture Result, see Table 4. When multiple sputa are collected on a single day, all results of each inoculation are used to establish the Culture Result.

For some analyses, solid and liquid culture will be analyzed separately. For these analyses, definitions in Table 4 will be used, considering only inoculations from that culture media.
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Table 4. Specimen Culture Result Classification for all inoculation results

Culture Result Classification	**Definition for visits when a single specimen (multiple media) is collected**	**Definition for visits when multiple specimens (multiple media) are collected**
Mtb Culture Positive	**Any inoculation from a single specimen is Mtb Positive**	**Any inoculation from any specimen collected on a single day is Mtb Positive**
Mtb Culture Negative	**All inoculations are Mtb Negative for a single specimen** **When at least one inoculation from a specimen is Mtb Negative and additional inoculation(s) is Contaminated** When at least one inoculation from a specimen is Mtb Negative and additional inoculation(s) is Unevaluable	**All inoculations from all specimens collected on a single day are Mtb Negative** **When at least one inoculation from all specimens collected on a single day is Mtb Negative and additional inoculation(s) is Unevaluable or Contaminated**
Culture Contaminated	**All inoculation results are Contaminated for a single specimen** When at least one inoculation from a single specimen is Contaminated and additional inoculation(s) is Unevaluable	**All inoculations from all specimens collected on a single day are Contaminated** **When at least one inoculation from all specimens collected on a single day is Contaminated and additional inoculation(s) is Unevaluable**
Culture Unevaluable	**All inoculation results from a single specimen are Unevaluable**	**All inoculations from all specimens collected on a single day are Unevaluable**
Unable to produce sputum	**Attempt to collect sputum is unsuccessful as participant is unable to produce sputum**	**All attempts to collect sputum is unsuccessful as participant is unable to produce sputum**

5 Efficacy Analyses

5.1 Study Populations

There will be five analysis groups, as follows:

5.1.1 Intention-to-Treat (ITT)

Includes all randomized participants.

5.1.2 Microbiologically Eligible

Includes the subset of Intention-to-Treat participants who, in addition, have culture confirmation of drug-susceptible tuberculosis as defined by section 4.3, and were not randomized in error as defined by section 4.2. Participants classified as 'not assessable' will be considered to have an unfavorable outcome in this analysis.

5.1.3 Assessable

Includes the subset of Microbiologically Eligible participants who, in addition, are not classified as 'not assessable'.

5.1.4 Adherent Per-Protocol (PP95)

Includes the subset of Assessable participants who, receive 95% of assigned treatment as defined in Section 4.4. Participants in the Assessable study population that do not complete adequate treatment for the reason of death or bacteriological treatment failure will be included in the PP95 analysis population provided they receive 95% of doses up to the time of treatment withdrawal. This will be the primary PP analysis population.

5.1.5 Adherent Per-Protocol (PP75)

Includes the subset of Assessable participants who, receive 75% of assigned treatment as defined in Section 4.4. Participants in the Assessable study population that do not complete adequate treatment for the reason of death or bacteriological treatment failure will be included in the PP75 analysis population provided they receive 75% of doses up to the time of treatment withdrawal. This is a supportive PP analysis population and is included for comparability with previous trials (particularly REMoxTB)

5.2 Analysis plan for the primary efficacy outcome

5.2.1 Co-primary efficacy analyses

The primary analyses of efficacy is based on the modified ITT populations: the Microbiologically Eligible and Assessable study populations. While an ITT analysis is commonly used for a superiority trial, it may not be sufficiently conservative for a non-inferiority trial since ITT analysis can bias toward no treatment difference. Both the International Conference on Harmonisation (ICH) E9 document of Statistical Principles for Clinical Trials[7] and the FDA document of Guidance for Industry Non-Inferiority Clinical Trials (REF) suggest looking at the ITT analysis cautiously for a non-inferiority trial and recommend considering PP analysis as equally important for a non-inferiority trial[8]. A PP analysis, however, does introduce bias through exclusions based on post-randomization exclusions[9] which is particularly problematic in a study that is not placebo-controlled. The PP analyses (PP95 and PP75) will therefore be considered secondary in the primary efficacy analyses.

There will be two co-primary efficacy analyses, one for the Microbiologically Eligible population and another for Assessable population. Non-inferiority must be demonstrated in both populations in order to declare non-inferiority for an intervention regimen.

For each, the comparison of unfavorable outcome as defined by protocol at primary efficacy endpoint for Regimen 1 (control regimen) versus Regimen 3 (2PHZM/2PHM) will be considered first, and, if non-inferiority criteria are met, then the comparison of Regimen 1 versus Regimen 2 (2PHZE/2PH) will be considered.
In the event that the arm of Regimen 3 is terminated for safety concerns in the interim monitoring, the comparison of Regimen1 versus Regimen 2 (2PHZE/2PH) will be carried out for co-primary efficacy analyses.
If Regimen 3 is not terminated for safety concerns in the interim monitoring and the non-inferiority criteria for Regimen 1 versus Regimen 3 at the primary endpoint are not met (see 5.2.1), then the comparison of Regimen 1 versus Regimen 2 at the primary endpoint will be considered as an exploratory efficacy analysis.

5.2.2 Non-inferiority test

The proportion of participants with unfavorable outcome can be estimated thus: if r_a and r_b are the proportions of unfavorable outcome for the two study arms (*a* = Regimen 1 and *b* = Regimen 2 or Regimen 3) and if δ is the non-inferiority margin (= 6.6%), then the statistical hypothesis is:

$$H_0 : r_b - r_a \geq \delta \quad \text{vs.} \quad H_1 : r_b - r_a < \delta$$

For a significance level of 0.05, the statistical test is equivalent to obtaining the **two-sided 95% confidence interval for the difference of $r_b - r_a$.** If the upper bound of the confidence interval is less than δ, then the non-inferiority of Regimen 2 or Regimen 3 to Regimen 1 with a significance level of 0.05 is established (equivalent to one-sided α=0.025).
The difference in proportion unfavorable will be calculated using a stratified analysis using Cochran-Mantel-Haenszel weights[10]. Randomization is stratified by site, HIV status and presence of cavitation. The analysis will be stratified by HIV status and presence of cavitation only. Inclusion of site as a factor in the stratified analysis will result in some groups with too few participants. The unstratified difference in proportions will also be presented, although the stratified difference will be considered primary.

5.2.3 Superiority test

Regimens that are shown to be non-inferior to control will also be assessed for superiority compared to control using a two-sided significance level of 0.05.

5.2.4 Secondary efficacy analyses

In additional secondary efficacy analyses, the primary efficacy endpoint will be assessed in the PP95 and PP75 populations, in addition to the full ITT population.

5.2.5 Tabulation of the primary outcome classification

Since the primary efficacy outcome is a composite of various components, the actual reason (component) for outcome will also be tabulated by treatment arm.
Participants will be classified by the first event (chronologically) that made the participant unfavorable and further sub-classified by the bacteriological status at the time that this outcome occurred (culture converted, never culture converted, bacteriological reversion, or bacteriological relapse).

5.2.6 Sub-group analyses

This primary efficacy analysis will be repeated in subgroups according to the following baseline factors (i.e. those present at enrollment or from study-specific samples collected for screening and baseline visits). For factors reliant on results from sputum samples, the results must be from the study laboratory of record. Categorical variables will be split by tertiles except where there is previous clinical justification for a different cut-off.

- HIV status
- Presence of cavitation on baseline chest radiograph
- Extent of cavitation on baseline chest radiograph
- Sex
- Weight
- BMI
- WHO scale smear quantification
- Solid culture colony count
- MGIT days to detection
- GeneXpert MTB/RIF Cycle Threshold
- Age
- Country of study center
- Smoking history
- History of diabetes
- Ethnicity and race

The test for an interaction between the covariate and treatment will be done using logistic regression comparing the model including the interaction term and the model with only marginal terms using the likelihood ratio test to evaluate the statistical significance of inclusion of the interaction term in the model.

5.2.7 Independent Endpoint Review Committee

An independent blinded endpoint review committee (ERC) will be formed to review individual primary efficacy outcome classification at the end of the trial in the event that the assessment of non-inferiority is marginal for one or both regimens.

Members of the ERC will be identified prior to the end of the trial, and will include a small number of experts with experience in conducting clinical trials for new treatments for TB who are not otherwise involved in the trial. The ERC may not be needed, but may be convened and asked to review individual primary efficacy outcome classification for a subset of patients under the following scenarios:

- if the upper bound of the 95% confidence interval of the difference in the proportion of unfavorable outcomes is within 2% of the non-inferiority margin of 6.6%, in other words between 4.6% and 8.6%, for either regimen as compared to control provided the regimen is shown to safe and well tolerated. This range includes scenarios when non-inferiority has been demonstrated (<6.6%) or just missed (>6.6%).
- if there are large number of outcomes (10% or more) that are based on limited bacteriology or limited data and the review by the ERC would be considered valuable in interpreting the results.

The ERC may be asked to review outcome classification in other situations.

The ERC will review the full bacteriological, clinical and baseline data for participants, while staying blinded to treatment allocation. The ERC will review all participants classified as unfavorable or not assessable in the primary efficacy outcome, although they would be expected to focus on the more complex cases and more straightforward cases (e.g. clear

bacteriological relapse) might be presented in summary form. The ERC will be asked to determine whether, based on the entirety of the data available, they would consider this participant to have had an unfavorable outcome of treatment or be in the process of having an unfavorable outcome of treatment and to quantify their confidence using a scale from 1 (very unlikely) to 4 (very likely). The ERC will be encouraged to come to consensus in each case. Any re-classification by the ERC will not replace the primary efficacy outcome defined based on the algorithm described in this analysis plan, but will be a sensitivity analysis used to supplement the primary efficacy results and provide further insight into whether the intervention regimens should be considered to have non-inferior efficacy.

A document further describing the terms of reference of the endpoint review committee will be prepared prior to the end of the trial.

5.2.8 Additional sensitivity analyses

The following additional sensitivity analyses will be conducted:

1. The primary efficacy analysis will be repeated in the Microbiologically Eligible study population where all participants classified as not assessable will be classified as favorable rather than unfavorable.
2. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations where participants taking any non-study anti-TB medications for more than 21 days for any reason (including secondary isoniazid preventative therapy) will instead be classified as unfavorable.
3. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations where participants taking any non-study anti-TB medications for more than 5 days for any reason (including secondary isoniazid preventative therapy) will instead be classified as unfavorable.
4. The primary efficacy analysis will be repeated with a modification to the definitions of 'Absence of Cure' using the following text to replace the paragraph numbered 1 in section 4.1.1. so that intervening negative cultures are ignored in the determination of absence of bacteriological cure:

   *A participant will be considered to have absence of bacteriological cure if he/she has a sputum sample, obtained at or after Week 17 and no later than the end of the Month 12 analysis visit window, that is Mtb Culture Positive (see section 4.7 for definitions of culture results) that is indistinguishable from the initial isolate (see separate sequencing plan for definitions), and this is confirmed by a second sample that is Mtb culture positive. A second confirmatory sample, on a different day (**irrespective of intervening Mtb Negative culture results)**, is required, as a single positive sputum culture result in isolation will not be considered absence of bacteriological cure. If results from strain analysis are inconclusive or unavailable, it will be assumed that strains were indistinguishable.*
5. The primary efficacy analysis will be repeated reclassifying all exogenous reinfections as unfavorable.
6. The primary efficacy analysis will be repeated considering only culture inoculation results from MGIT liquid media and ignoring any culture inoculation results from solid media.
7. The primary efficacy analysis will be repeated considering only culture inoculation results from solid media and ignoring any culture inoculation results from MGIT liquid media.

8. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations excluding additionally participants for whom none of screening or baseline study visit sputum cultures are Mtb Positive (week 2 sputum cultures will not be used for determining late exclusions).
9. The primary efficacy analysis will be repeated in the Microbiologically Eligible and Assessable study populations including all participants classified as randomized in error. For such patients, the classification of the outcome will follow the algorithm in section 4.1.

5.2.9 Bayesian analysis of non-inferiority

A Bayesian analysis of non-inferiority provides a more informative interpretation of the trial results by providing an estimate of the probability that the interventions regimens have efficacy not much worse than the control regimen for different thresholds of what might be considered 'not much worse'. Following methods described previously[11], Bayesian binomial regression will be used to estimate the distribution of the difference in the proportion of unfavorable outcomes between each intervention arm and the control arm, in each analysis population considering both non-informative and informative priors. These methods have also been used in secondary analyses of the STREAM Stage 1 trial (NCT02409290).

5.3 Analysis plan for secondary efficacy outcomes

5.3.1 TB disease-free survival at eighteen months after study treatment assignment

The primary efficacy endpoint for the trial is TB disease-free survival twelve months after study treatment assignment. For that primary analysis (described above), only data up to and including the 12 month visit window will be used, see section 4.1 above. Participants are followed for eighteen months from start of treatment and an important secondary efficacy outcome is therefore TB disease-free survival eighteen months after study treatment assignment.

For this analysis (TB disease-free survival eighteen months after study treatment assignment), the same definitions for the primary efficacy outcome in section 4.1 will be used with the exception that the Month 18 visit will be used in the definitions in place of the Month 12 visit. All the same analyses for the primary efficacy analysis described in section 5.2 will be repeated using this Month 18 endpoint.

5.3.2 Time to stable culture conversion

Time to stable culture conversion is defined as the time from randomization to the first of two consecutive Mtb Culture Negative results, collected on separate days without an intervening Mtb Culture Positive, and processed at the study lab of record. Participants that never achieve culture conversion will be censored at the date of collection of sputum that yielded their last negative or positive culture result.

Time to stable culture conversion will be analyzed separately for cultures from solid and liquid culture media. The Microbiologically Eligible study population will be used for these analyses. Median time to stable culture conversion will be calculated for each regimen. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.

The equality of survivor functions for time to stable culture conversion will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.
The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.
Even when Kaplan-Meier curves of time to culture conversion have been shown to diverge in the presence of an effective drug (such as bedaquiline), they tend to converge later in followup potentially violating the assumption of proportional hazards. In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.
The analyses above of time to sputum culture conversion will be repeated as follows:

1. With the alternative definition as time from randomisation to the first Mtb Negative culture from sputa processed at the study lab of record (without the need for a second negative culture to confirm).
2. Including only participants for whom 75% of more of culture results from sputum samples collected between randomization and week 12 are evaluable.

The Kaplan-Meier estimator will be used to calculate the proportion of participants with 95% confidence interval who are culture negative after 8 weeks and after 12 weeks.

5.3.3 Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection

The Microbiologically Eligible study population will be used for these analyses.
Speed of decline of MGIT days to detection will be analyzed using mixed effect non-linear regression comparing different parametric models, and taking account of the latest research and other relevant published studies. Parametric models for modelling the quantitative decline in viable bacilli used to date have included bi-exponential[12], hyperbolic tangent function[13] and surge functions[14].

5.3.4 Time to unfavorable efficacy outcome

Time to unfavorable efficacy outcome is defined as the time from randomization to the first event that results in the definition of an unfavorable efficacy outcome for that participant.
The time will be analyzed first using the primary efficacy outcome (using data from randomization to 12 months) and also using the secondary efficacy outcome at 18 months (as defined in section 5.3.1)
Participants that do not achieve culture conversion (i.e. fail to have 2 consecutive culture negative results), and have not otherwise been called unfavorable, will be called unfavorable at the date of the last visit when a culture positive result was obtained.
These analyses will be repeated for the Microbiologically Eligible, PP95 and PP75 study populations.
Participants classified as favorable or not assessable will be censored in this analysis at the date of collection of sputum that yielded their last negative culture result.
Median time to unfavorable outcome will be calculated for each regimen. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.
The equality of survivor functions will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.

6 Safety Analyses

6.1 Analysis population

All safety analyses will be conducted using the Safety Analysis Population which includes all participants randomized that took at least one dose of the allocated study regimen.

6.2 Primary safety analysis

The primary safety outcome is the proportion of participants with grade 3 or higher adverse events during the study treatment phase (defined above in section 4.6).

The difference in proportion of participants with a grade 3 or higher adverse event during study treatment phase between each of Regimens 2 and 3 compared to Regimen 1 with corresponding two-sided 95% confidence intervals will be estimated using the same methods as for the primary efficacy analysis (section 5.2.2).

It is hypothesized that the rifapentine regimens will have safety at least as good as the control regimen, but no non-inferiority margin has been pre-specified. The objective is to estimate the difference between regimens and describe the precision in this estimate (using 95% confidence intervals).

The primary safety analysis will also be repeated in the Microbiologically Eligible and Assessable analysis populations.

6.3 Secondary safety analyses

6.3.1 Treatment-related grade 3 or higher adverse events

The primary safety analysis will be repeated considering only the subset of grade 3 or higher adverse events during study treatment phase that are considered at least possibly related to study treatment.

6.3.2 Tolerability

Tolerability of the regimen is evaluated using the outcome of discontinuation of assigned treatment for a reason other than microbiological ineligibility. For this reason, participants excluded from the microbiologically eligible study population will also be excluded from this analysis.

The proportion of participants discontinuing assigned treatment will be tabulated by regimen, by week of discontinuation after date of start of treatment, and by cause of discontinuation (e.g. adverse event, withdrawal of consent, treatment failure).

6.3.3 All-cause mortality during treatment or follow-up

All-cause mortality includes all deaths from any cause during treatment or follow-up up to the end of the Month 18 visit window.

The number of participants who die during treatment and follow-up will be tabulated by regimen with listings of primary cause of death.

Median survival time will be calculated for each regimen. Participants that do not die will be censored at the date last known to be alive. A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors of HIV status and presence of cavitation.

The equality of survivor functions will be compared using a (Wilcoxon) Log rank test, stratified by HIV status and presence of cavitation. Regimen 2 and Regimen 3 will each be compared with the control Regimen 1.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals and by reviewing the log minus log survival plot (against log time) after fitting the Cox Proportional Hazards model.

6.3.4 Serious adverse events (SAEs)

The proportion of participants with an SAE during study treatment phase will be tabulated by regimen, MedDRA system organ class (SOC), MedDRA preferred term (PT) and severity grade. The proportion of participants with a Serious Adverse Reactions (SARs) and Suspected Unexpected SARs (SUSARs) during study treatment phase will also be tabulated by regimen and severity grade.

6.3.5 All grade adverse events (AEs)

The proportion of participants with any AE during study treatment phase will be tabulated by regimen, and relatedness to allocated regimen, MedDRA system organ class, MedDRA preferred term and severity grade.

6.3.6 Laboratory safety parameters

Laboratory safety parameters will be summarized by regimen and study visit (scaled by upper or lower limit of normal, ULN) including a summary of the highest (or lowest) measurement recorded at any visit. Summaries of drug induced liver injury (DILI) will be included using the published definition from secondary analyses of the REMoxTB trial[15] (ALT > 5x ULN and ALT > 3x ULN and total bilirubin >2 x ULN), ATS definitions and using Hy's law.
These summaries will be repeated limiting to measurements only during the study treatment phase.

6.3.7 Acquired drug resistance

Acquired drug resistance will be summarized by regimen, distinguishing between resistance acquired at time of treatment failure or relapse following study treatment and resistance acquired while on a non-study retreatment regimen.

7 Data summaries

7.1 Recruitment and baseline characteristics

7.1.1 Recruitment, screening, & eligibility

The number of participants screened and randomized will be tabulated by center and regimen. The number of participants who were not enrolled will be presented by center.

7.1.2 Exclusions from analysis

The number of participants excluded from each of the study populations will be tabulated by regimen and by reason for exclusion.

7.1.3 Baseline characteristics

Baseline characteristics will be tabulated by regimen. Characteristics will include sex, age, ethnicity, weight, BMI, whether underweight (BMI <18.5 kg/m^2), race, ethnicity, cavitation, radiographic severity of disease, Karnofsky score, and laboratory parameters such as, HIV status, CD4 count (if applicable), smoking status, smear and culture grading, serum albumin, hemoglobin, and time to positivity on MGIT. The baseline characteristics table will be repeated for each of study populations.

7.2 Adherence to study medications

The protocol requires at least 5 weekly doses to be provided by directly observed therapy (DOT) (i.e. participants should receive ~71% of study doses as DOT). The rest of doses are allowed to be self-administered (SAT).
Treatment adherence will be summarized by regimen first considering only DOT doses and also considering DOT and SAT doses.

7.3 Concomitant medications

Use of concomitant medications will be summarized by treatment arm considering separately 1. Non-TB and Non-antiretroviral medications, 2. Anti-retroviral medications, and 3. Additional non-study TB medications.

7.4 Retention, participant disposition, and description of follow-up

Completion of treatment and completion of scheduled follow-up will be summarized by regimen including reasons for failure to complete treatment and follow-up.

8 Other Analyses

8.1 Risk Factor Analysis

Univariate and multivariate analyses will be carried out to assess the association of unfavorable outcomes and clinical and demographic variables using Cox regression (time to event endpoints) and logistic regression (binary endpoints). Variables included as potential risk factors include baseline data in addition to on-treatment data such as time to culture conversion and weight gain. The most parsimonious model that contains significant variables with $p<0.05$ will be used to identify risk factors. The interactions between risk factors and treatment regimens will be examined to evaluate variations of different risk factors among treatment regimens.

8.2 Efavirenz Pharmacokinetics Analysis

Efavirenz pharmacokinetics will be evaluated in the first 31 and then a total of 90 participants from each of two groups of participants randomized to treatment regimen 2 or 3 of the study and receiving efavirenz based ART. The analysis plan for this is described in a separate statistical analysis plan (TBTC Study 31 Efavirenz Pharmacokinetic Statistical Analysis Plan).

9 Interim monitoring by the Data and Safety Monitoring Board (DSMB)

Interim analyses will be provided to the DSMB for monitoring the efficacy and safety and for the DSMB to review other matters occurring during the trial. Based on the interim results, the DSMB may recommend early closure of any experimental arm or the trial if the interim evidence is sufficiently strong that one of the trial interventions is clearly indicated or clearly contraindicated because of a net difference in efficacy, safety, or tolerability.

If the evidence provides proof beyond reasonable doubt that there is a clear and meaningful benefit or harm of a given regimen, the DSMB may recommend termination of one of the treatment arms or termination of the study.

9.1 Monitoring for efficacy

Two interim analyses for the primary efficacy outcome are planned. To provide guidance to continue, adjust or stop the trial, the two interim analyses will be carried out after 40% and 60% of participants, respectively, reach 12 months post-randomization, and allowing for up to an additional four months for reporting of all culture results. The primary efficacy outcome will be conducted according to the statistical analysis plan described in section 5.2 with the analysis populations described therein.

There will be no ordering of hypotheses in the interim analyses.

The Haybittle-Peto stopping boundaries are to be used for the interim analyses. Following the Haybittle-Peto rule in monitoring the efficacy, the statistical criterion for the differences between arms is set at the 0.1% level of statistical significance in each of the two interim analyses. If any arm is shown to have superior efficacy to the control arm at the 0.1% level of statistical significance (i.e. using 99.9% confidence intervals), the DSMB should consider whether it would be appropriate to terminate some arms or the whole trial, taking into account other efficacy and safety analyses.

9.2 Monitoring for safety

The DSMB will convene approximately annually, or more often as needed, to review safety data. The safety monitoring in the interim analyses will focus on the comparison of the proportion of participants with adverse events in each treatment arm, with a particular focus on the primary safety endpoint. Concerns about the extent and type of adverse events observed may lead to early termination of the trial when the DSMB judges that the potential benefits of the new regimens are unlikely to outweigh the risks. In other cases, the DSMB may recommend measures short of termination that may reduce the risk of adverse events. For example, modifying the inclusion criteria if the risks are concentrated in a particular subgroup or instituting screening procedures that could identify those at increased risk of a particular adverse event.

10 Version history

Version 1.0	12 December 2018

10.1 List of changes from version 1.0

11 References

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