A Phase 2a Study of the Early Bactericidal Activity of Rifampin in Combination with Meropenem plus Amoxicillin/Clavulanate Among Adults with Rifampin-Resistant or Rifampin-Susceptible Pulmonary Tuberculosis

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1 PROTOCOL SUMMARY

Title: A Phase 2a Study of the Early Bactericidal Activity of Rifampin in Combination with Meropenem plus Amoxicillin/Clavulanate Among Adults with Rifampin-Resistant or Rifampin-Susceptible Pulmonary Tuberculosis

Goal: The overall goal of this exploratory proof-of-concept study is to determine whether, in participants with pulmonary tuberculosis caused by *M. tuberculosis* with or without rifampin resistance-conferring *rpoB* mutations, the combination of meropenem and amoxicillin/clavulanate *with rifampin* has greater early bactericidal activity (EBA) than the combination of meropenem and amoxicillin/clavulanate *without rifampin*.

Phase: 2a

Design: Open-label, randomized clinical trial examining the bactericidal activity of meropenem plus amoxicillin/clavulanate with versus without rifampin

Population: Adults with sputum smear positive pulmonary tuberculosis caused by *M. tuberculosis* with or without rifampin resistance-conferring rpoB mutations

Number of sites: One enrollment site located in South Africa

Study duration: Approximately 30 days for each participant

Intervention:

	Regimen	**Number per arm**	**Regimen components**		
Rifampin resistant	A	24	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	B	16	-	Meropenem 2 grams thrice daily	Amx/Clv
Rifampin susceptible	C	12	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	D	12	-	Meropenem 2 grams thrice daily	Amx/Clv
	E	12	-	Meropenem *1 gram thrice daily*	Amx/Clv
	F	12	-	Meropenem *3 grams once daily*	Amx/Clv

Abbreviation:Amx/Clv, amoxicillin/clavulanate

Objectives: *Primary*

- Among participants with pulmonary TB caused by *M. tuberculosis* with or without rifampin resistance-conferring *rpoB* mutations: to estimate the 14-day EBA, based on colony forming unit counts, of the combination of meropenem and amoxicillin/clavulanate, without versus with rifampin

- To compare the magnitude of the effect on 14-day EBA, based on colony forming units, of the addition of rifampin to meropenem and amoxicillin/clavulanate in

participants with pulmonary TB caused by *M. tuberculosis* with vs. without rifampin resistance-conferring *rpoB* mutations.

- To determine the association between the area under the concentration-time curve divided by minimum inhibitory concentration (AUC/MIC) of rifampin and the antimycobacterial activity of the study treatments, adjusting for other factors known to affect treatment response

- To characterize the exposure-response relationship of meropenem (in combination with amoxicillin/clavulanate) on 14-day EBA in participants with pulmonary TB

Secondary

- To describe the safety and tolerability of the study regimens, administered for 14 days

- Estimate the 14-day antimycobacterial activity, based on liquid culture time-to-positivity, of the study treatments

- Determine the steady state pharmacokinetics of rifampin and meropenem

- Determine the MICs of the study drugs, alone and in combination, for participant's *M. tuberculosis* isolates

- Estimate the 2-day antimycobacterial activity, based on colony forming unit counts and also based on liquid culture time-to-positivity

Endpoints:

Primary

- Mean daily fall in $\log_{10}$CFU of *M. tuberculosis* per mL sputum over14 days of treatment ($EBA_{0\text{-}14}$(CFU)

- Steady state PK and PK/PD parameters (including $AUC_{0\text{-}24}$,maximum concentration (C_{max}), MIC against *M. tuberculosis*; time above MIC) for rifampin and meropenem

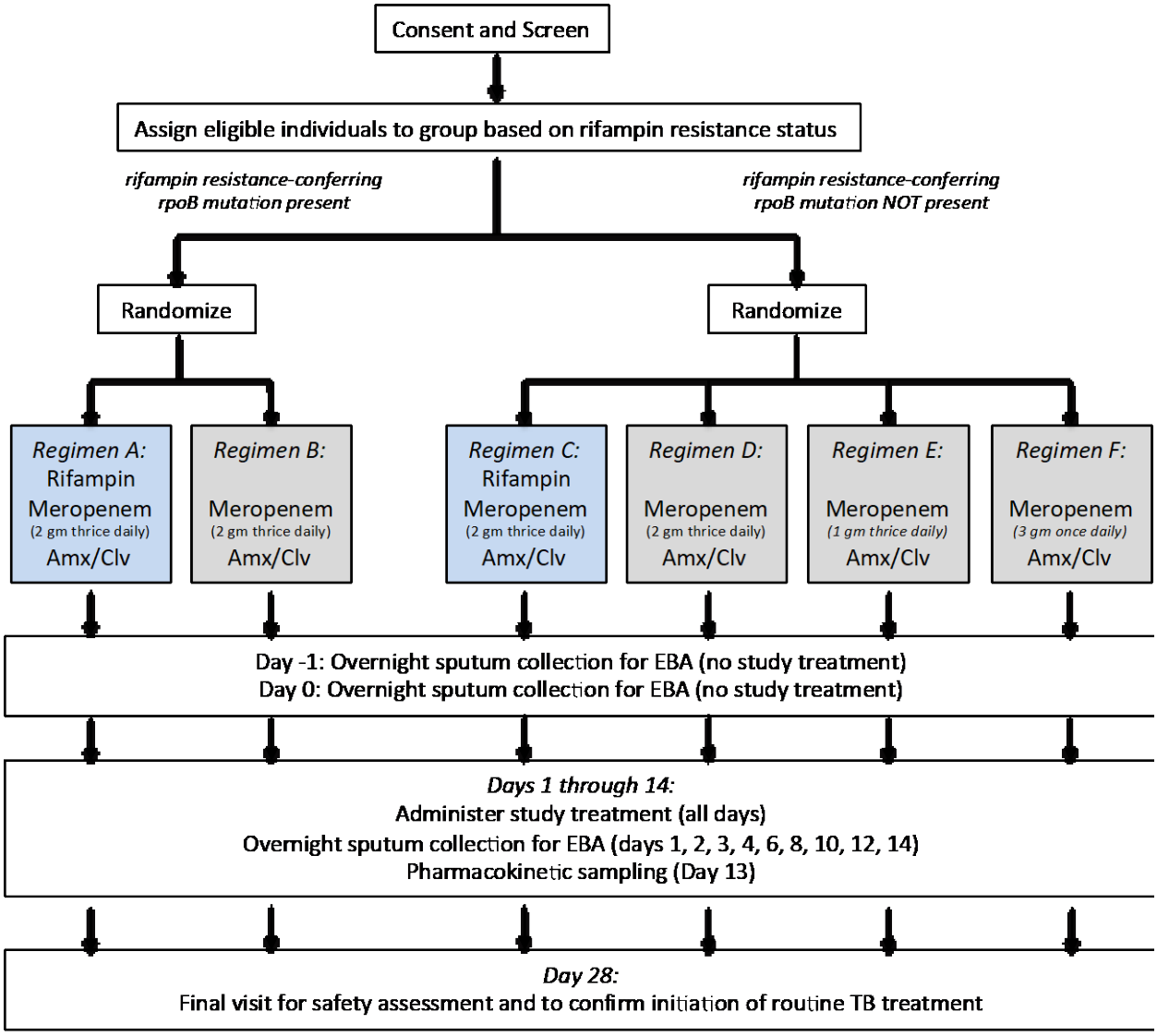
Secondary

- Grade 2 or higher drug-related adverse clinical or laboratory events

- Change in time-to-positivity in MGIT liquid media over 14 days of treatment ($EBA_{0\text{-}14}$(TTP))

- Mean daily fall in $\log_{10}$CFU of *M. tuberculosis* per mL sputum over2 days of treatment ($EBA_{0\text{-}2}$(CFU)

Schema



Notes:
Rifampin dose 20 mg/kg once daily by mouth
Meropenem administered by intravenous infusion
Amx/Clv=Amoxicillin/Clavulanate, dose 500 mg/125 mg thrice daily by mouth
EBA=early bactericidal activity

2 STUDY GOAL AND OBJECTIVES

2.1 Goal

The overall goal of this exploratory proof-of-concept study is to determine whether, in participants with pulmonary tuberculosis caused by *M. tuberculosis* with or without rifampin resistance-conferring *rpoB* mutations, the combination of meropenem and amoxicillin/clavulanate *with rifampin* has greater early bactericidal activity (EBA) than the combination of meropenem and amoxicillin/clavulanate *without rifampin*.

2.2 Primary Objectives

2.2.1 Among participants with pulmonary TB caused by *M. tuberculosis* with or without rifampin resistance-conferring *rpoB* mutations: to estimate the 14-day EBA, based on colony forming unit counts, of the combination of meropenem and amoxicillin/clavulanate, without versus with rifampin

2.2.2 To compare the magnitude of the effect on 14-day EBA, based on colony forming units, of the addition of rifampin to meropenem and amoxicillin/clavulanate in participants with pulmonary TB caused by *M. tuberculosis* with vs. without rifampin resistance-conferring *rpoB* mutations.

2.2.3 To determine the association between the area under the concentration-time curve divided by minimum inhibitory concentration (AUC/MIC) of rifampin and the antimycobacterial activity of the study treatments, adjusting for other factors known to affect treatment response

2.2.4 To characterize the exposure-response relationship of meropenem (in combination with amoxicillin/clavulanate) on 14-day EBA in participants with pulmonary TB

2.3 Secondary Objectives

2.3.1 To describe the safety and tolerability of the combination of the study regimens, administered for 14 days

2.3.2 Estimate the 14-day antimycobacterial activity, based on liquid culture time-to-positivity, of the study treatments

2.3.3 Determine the steady state pharmacokinetics of rifampin and meropenem

2.3.4 Determine the MICs of the study drugs, alone and in combination, for participant's *M. tuberculosis* isolates

2.3.5 Estimate the 2-day antimycobacterial activity, based on colony forming unit counts and also based on liquid culture time-to-positivity

3 INTRODUCTION

3.1 Scientific background

3.1.1 Drug-resistant tuberculosis (TB) as a global health problem: the need for novel treatment regimens

Mycobacterium tuberculosis has just surpassed HIV as the single greatest killer by an infectious pathogen on the planet. The World Health Organization (WHO) estimates that over 9 million new cases and 1.54 million deaths due to tuberculosis (TB) occurred in 2013 [WHO 2013a and 2013b]. Multidrug-resistant (MDR) TB,i.e. TB resistant to isoniazid and rifampin, is a growing public health threat, with an estimated 480,000 cases in 2013, and extensively drug-resistant (XDR) TB, i.e. TB resistant to isoniazid, rifampin, fluoroquinolones, and injectable anti-TB drugs, has been found in every country in which it has been sought [WHO 2013b]. TB resistant to all currently available anti-TB drugs is now a reality and threatens a return to the pre-antibiotic era [Klopper 2013; Loewenberg 2012; Slomski 2013; Udwadia 2012]. Therapeutic options for highly drug-resistant TB are limited in availability, acceptability and efficacy. For example, only 1 in 5 patients diagnosed with MDR-TB is started on treatment [WHO 2013b]. Current treatment for MDR-TB requires 18-24 months of multidrug therapy, including at least 6 months of an injectable agent, and yet is successful in only 48% of patients [WHO 2011; Ahuja 2012]. For select patients, a seven drug 9-12 month "short course" regimen is now available in some settings [WHO 2016]. Current regimens, whether short course or standard duration, for the treatment of highly drug-resistant TB are poorly-tolerated and have significant toxicities. Several drugs with novel mechanisms of action recently have been approved or are in advanced phase clinical trials, but the drug pipeline is not robust and new drugs must be combined with other effective antituberculosis drugs to prevent acquired drug resistance and optimize the likelihood of cure. Therefore improved treatments of drug-resistant TB are critical to improve patient cure rates and to alleviate suffering related to the common severe side effects of standard drug-resistant TB regimens.

3.1.2 Optimizing the use of existing antibiotics is a rational strategy for improving the treatment of drug-resistant TB.

There are two approaches that can be taken to developing new treatments for TB and drug-resistant TB. The first is the development of new chemical entities with antituberculosis activity but without cross-resistance to existing antibiotics. Bedaquiline and delamanid are examples of new chemical entities with novel mechanisms of action. The second approach is the optimization of the use of existing drugs or combinations of drugs. By virtue of rifampin's unique treatment-shortening effect in modern short-course TB regimens, resistance of an infecting *M. tuberculosis* strain to rifampin is primarily responsible for the prolonged treatment duration required to treat MDR-TB. Strategies that restore rifampin susceptibility, even partially, may have important treatment-shortening effects on MDR-TB regimens.

3.1.3 M. tuberculosis has intrinsic resistance to β-lactam antibiotics

β-lactam antibiotics inhibit bacterial cell wall synthesis by binding to and consequently inactivating the transpeptidases that cross-link peptidoglycan chains to form the cell wall. The antibacterial activity of penicillins and cephalosporins are mediated mainly through their inactivation of classical D,D-transpeptidases. Historically, β-lactam antibiotics have seldom been considered for TB treatment due to the perception that *M. tuberculosis* is intrinsically resistant to β-lactams due to the constitutive production of the chromosomally-encoded broad-spectrum β-lactamase, BlaC.

3.1.4 Clavulanate stably inactivates M. tuberculosis BlaC β-lactamase

Clavulanate is a β-lactam drug that functions as a β-lactamase inhibitor. Clavulanate itself has negligible intrinsic antimicrobial activity, but it functions as a suicide inhibitor of β-lactamases by covalently bonding to the active site of bacterially-encoded β-lactamases, thereby inactivating those enzymes and restoring the antimicrobial activity of β-lactam antibiotics. Clavulanate is marketed in combination with amoxicillin and also in combination with ticarcillin for the treatment of certain bacterial infections. Clavulanate has been

shown to irreversibly inhibit the *M. tuberculosis* BlaC β-lactamase. Clavulanate reacts with the BlaC β-lactamase quickly to form hydrolytically stable, inactive forms of the enzyme [Hugonnet 2007]. Tazobactam and sulbactam also inhibit the BlaC gene product, but the covalent adduct generated is unstable and is hydrolyzed. Thus, among the FDA-approved β-lactamase inhibitors, clavulanate appears to be the most promising with respect to activity against *M. tuberculosis*.

3.1.5 Carbapenems, which are relatively poor substrates for the M. tuberculosis BlaCβ-lactamase, have intrinsic activity against M. tuberculosis in vitro; this activity is potentiated by clavulanate and further potentiated by amoxicillin/clavulanate

Carbapenems are members of the β-lactam class of antibiotics. However, unlike penicillins and cephalosporins, carbapenems are relatively poor substrates for the *M. tuberculosis* BlaC β-lactamase. Meropenem, a carbapenem approved by the FDA for use in certain bacterial infections, has activity against *M. tuberculosis* in vitro, and this activity is potentiated by clavulanate [Hugonnet 2009, Kaushik 2015]. The combination of meropenem and clavulanate has an observed MIC of < 1 ug/mL against *M. tuberculosis*, sterilizes aerobically grown cultures within 14 days, and has inhibitory activity against anaerobically grown cultures used to model a persistent state of *M. tuberculosis*[Hugonnet 2009].Clavulanate is not available for human use except in combination with amoxicillin or ticarcillin. Gonzalo and Drobniewski recently studied 28 *M. tuberculosis* strains including MDR and XDR strains to assess if meropenem/clavulanate was active at concentrations achievable in vivo and to determine whether there was inhibition of meropenem/clavulanate activity in the presence of amoxicillin. They found that the addition of clavulanate to meropenem reduced the MIC of meropenem by an average of over 1.8 dilutions, while the addition of amoxicillin to meropenem/clavulanate reduced the MIC of meropenem by an average of 3.2 dilutions [Gonzalo 2013]. The mechanism is not clear, but may be through inhibition of different transpeptidases by meropenem vs. amoxicillin. Thus, in vitro studies provide rationale for further study of the combination of meropenem and amoxicillin/clavulanate for TB treatment.

3.1.6 Carbapenems inhibit non-classical L,D-transpeptidases that are critical for M. tuberculosis cell wall biosynthesis and bacterial persistence

Until recently it was thought that D,D-transpeptidases (penicillin binding proteins) were the only enzymes involved in the final step of peptidoglycan cell wall biosynthesis. However, it was recently shown that *M. tuberculosis* contains L,D-transpeptidases that catalyzes3->3 linkages (as opposed to the 4->3 linkages catalyzed by classical D,D-transpeptidases) and are critical for cell wall biosynthesis[Gupta 2010; Erdemli 2012; Schoonmaker 2014]. In *M. tuberculosis* the majority of transpeptide linkages in the peptidoglycan are of the 3->3 type. Moreover, the *M. tuberculosis*L,D-transpeptidase is expressed as the organism enters the persistent state, with corresponding changes in the structure of the peptidoglycan [Lavollay 2008]. Loss of L,D-transpeptidase activity through mutation leads to reduced survival of *M. tuberculosis* during the chronic phase of infection in mice and increased susceptibility to amoxicillin/clavulanate[Gupta 2010; Schoonmaker 2014].Thus, the *M. tuberculosis* L,D-transpeptidases are an attractive target for drug development.

3.1.7 The combination of meropenem plus amoxicillin/clavulanate has antituberculosis activity in humans

The combination of meropenem plus amoxicillin/clavulanate has entered clinical practice for the treatment of highly drug-resistant TB based on the above in vitro data, the general safety and tolerability of these three drugs, and the paucity of other therapeutic options. To date, anecdotal reports and small case series support the efficacy and safety/tolerability of meropenem plus amoxicillin/clavulanate in combination with other agents in salvage regimens for drug-resistant TB [Yew 1995, Dauby 2011; Paven 2012; Palmero 2015, De Lorenzo 2013]. De Lorenzo and colleagues reported on an observational case-control study of 37 MDR/XDR-TB patients treated with meropenem plus amoxicillin/clavulanate in addition to a linezolid-containing regimen (cases) and 61 controls treated with a linezolid-containing regimen [De Lorenzo 2013].

Five of 37 (13.5%) of cases had adverse events potentially attributed to meropenem/amoxicillin/clavulanate. Two of these five cases experienced elevated liver transaminases - one patient had successful rechallenge with meropenem/amoxicillin/clavulanate and was able to continue this combination without problems, while the other patient experienced worsening of transaminases on re-challenge and was discontinued from meropenem/amoxicillin/clavulanate. The three other cases experienced diarrhea that did not require drug withdrawal. In spite of the higher clinical severity of the case group treated with meropenem plus amoxicillin/clavulanate, at 3 months the case group had a higher proportion of sputum smear converters than controls (28/32 [87.5%] vs. 9/16 [56.3%], p=0.02) and a higher proportion of culture converters (31/37 [83.8%] vs. 15/24 [62.5%], p=0.06).

Diacon and colleagues recently conducted a randomized controlled study of the 14-day early bactericidal activity of meropenem plus amoxicillin/clavulanate in adults with newly diagnosed sputum smear-positive drug-susceptible pulmonary TB[Diacon, 2016]. Participants randomized to the meropenem plus amoxicillin/clavulanate arm received meropenem 2 grams thrice daily by intravenous bolus, with amoxicillin 500 mg/clavulanate 125 mg administered orally thrice daily, for 14 days. Participants randomized to the control arm received standard antituberculosis therapy with daily oral isoniazid, rifampin, pyrazinamide, and ethambutol according to South African National Treatment Programme guidelines. Meropenem plus amoxicillin/clavulanate reduced the mean mycobacterial load by 0.11 (95% CI 0.09 to 0.13) $\log_{10}$colony forming units per mL of sputum per day and thereby exhibited robust 14-day early bactericidal activity (reference control regimen reduced mean mycobacterial load by 0.17 (95% CI 0.15 to 0.19)). Mild diarrhea that did not require dose adjustment was the most common adverse event among participants receiving meropenem plus amoxicillin/clavulanate regimen (10/15 [67%] participants). Despite proof-of-concept having been achieved, thrice daily intravenous meropenem administration is unlikely to be feasible in most settings of tuberculosis clinical care. Less frequent dosing of meropenem or another carbapenem suitable for intramuscular injection would increase feasibility significantly. A carbapenem formulated for oral administration would have the greatest impact on feasibility provided adequate drug exposures can be reached. To this end, Diacon et al also randomized participants in another arm of the same trial to receive faropenem sodium 600 mg with amoxicillin 500 mg/clavulanate 125 mg, each administered orally thrice daily, for 14 days. In contrast to meropenem, faropenem plus amoxicillin/clavulanate did not reduce the mean mycobacterial load at all (0.00, 95% CI 0.09 to 0.13 $\log_{10}$ colony forming units per mL of sputum per day). A better understanding of the pharmacokinetic drivers of activity and the optimal as well as minimal pharmacokinetic targets may inform the development of more feasible meropenem dosing strategies in the short term, as well as appropriate dosing strategies for novel oral carbapenems used for tuberculosis treatment in the medium and longer term.

3.1.8 The Combination of a Carbapenem plus Beta-Lactamase Inhibitor Restores the Activity of Rifampin Against 'Rifampin-Resistant' M. tuberculosis in vitro

Lamichhane and colleagues recently conducted a series of studies aimed at establishing whether carbapenems plus isoniazid or rifampin exhibited synergy, antagonism, or indifference in vitro against *M. tuberculosis* [Kaushik 2015]. Using checkerboard titration assays, the combination of meropenem and rifampin exhibited synergy against drug-susceptible *M. tuberculosis* strains, with a fractional inhibitory concentration (FIC) index of 0.4. Moreover, the MIC of rifampin against multiple rifampin-resistant *M. tuberculosis* strains was at least 10-100 times lower in the presence of a sub-inhibitory concentration of meropenem (Table 1).

Table 1. Representative growth profile for *M. tuberculosis* strain with rifampin MIC_{90} > 1 µg/mL
(-) indicates no visible growth; (+++) indicates growth equivalent to that of *M. tuberculosis* grown in broth alone.

[Rifampin] (µg/mL)	1.0	0.5	0.25	0.12	0.063	0.031	0.016	0
Meropenem 5 µg/mL	-	-	-	-	-	+	++	+++
No meropenem	+++	+++	+++	+++	+++	+++	+++	+++

The rifampin and meropenem concentrations are readily achievable clinically. For example a 600 mg oral dose of rifampin typically achieves a free drug level of approximately 1.5 µg/mL.

3.2 Study rationale

This is a proof-of-concept study to determine whether, in humans infected with *M. tuberculosis* that is resistant or susceptible to rifampin based on conventional drug susceptibility testing, the combination of meropenem, amoxicillin/clavulanate, and rifampin has activity that is sufficiently promising to proceed with further drug development along these lines. Rifampin has an incompletely understood but critical role in eradication of *M. tuberculosis* persisters and consequently the shortening of the duration of treatment for 'rifampin susceptible' TB. For MDR-/XDR-TB, the ability to recoup rifampin's antituberculosis activity through rational combination with a carbapenem and a β-lactamase inhibitor with or without amoxicillin could transform the treatment of this disease.

This proof-of-concept study is designed such that a negative outcome would refute the hypothesis that the combination of a carbapenem and amoxicillin/clavulanate with rifampin will have greater activity than either component alone against *M. tuberculosis* strains having MICs in the range considered resistant to rifampin. A positive study outcome would catalyze further research to identify optimal dosing strategies for all regimen components as well as development of carbapenems optimized for TB treatment with respect to targets of activity, stability against hydrolysis, and oral formulation.

The study hypothesis cannot be tested satisfactorily in traditional animal models of tuberculosis chemotherapy due to the rapid inactivation of carbapenems (as well as other beta-lactams) by dehydropeptidases that are expressed at high levels in mouse, rabbit, and guinea pig tissues. However, all of the study drugs are FDA-approved for various infectious disease indications, are in routine clinical use, and have good safety profiles, such that proceeding with the proposed clinical trial based on in vitro data is justified.

This study will also characterize the relationship between meropenem exposure (in combination with amoxicillin/clavulanate) and early bactericidal activity in order to identify the pharmacokinetic drivers of activity and pharmacokinetic targets for desired effects. This will inform the identification of more feasible meropenem dosing strategies in the near term, as well as the dose selection for novel oral carbapenems that may be available for tuberculosis treatment in the future. The proportion of the dosing interval for which free drug concentrations exceed MIC ($T_{>MIC}$) is the PK/PD parameter most closely correlated with efficacy of carbapenems against common fast-growing bacteria such as Enterobacteriaceae that cause infections for which meropenem is currently approved. A commonly accepted target for efficacy in these infections is 40% $T_{>MIC}$, which requires multiple daily doses to achieve. Whether this PK/PD parameter and target value is optimal for carbapenem treatment of infections with *M. tuberculosis*, which has a much longer doubling time, is unknown. In the trial by Diacon et al, meropenem 2 grams thrice daily plus amoxicillin/clavulanate

resulted in a median $T_{>MIC}$ of 76% (90% CI: 66-93) whereas faropenem sodium 600 mg thrice daily plus amoxicillin/clavulanate resulted in $T_{>MIC}$ of 13% (90% CI: 0-33), indicating that if $T_{>MIC}$ is the single parameter most strongly linked to efficacy in tuberculosis, then the target for bactericidal effect is between 13% and 76%, and lower and/or less frequent doses (or use of oral carbapenems with lower bioavailability) may still have significant efficacy. If $T_{>MIC}$ is not the efficacy-linked PK/PD parameter, less frequent administration of the same total dose is likely to remain equally effective.

3.3 Rationale for selection of doses and regimens

For this proof-of-concept clinical trial that is based on in vitro data and the recently demonstrated EBA of the combination of meropenem with amoxicillin/clavulanate, the selection of regimens and study drug doses aims to balance answering the key scientific question expeditiously and reducing risk. For participants with drug-resistant TB, the number of regimens has been restricted intentionally to the minimum required to determine whether or not, and to what degree, rifampin efficacy can be restored by combining high-dose rifampin with a potent carbapenem while reducing as best as possible the potential risk to study participants (in whom initiation of definitive treatment for tuberculosis will be delayed) and to study staff (since some participants will have drug-resistant TB). Accordingly, the dose of meropenem (2 grams every 8 hours) is that which has already been shown to have measurable, significant activity against *M. tuberculosis* when administered in combination with amoxicillin/clavulanate to patients with TB; the dose of 2 grams every 8 hours has regulatory approval for non-TB indications. The dose of rifampin, 20 mg/kg daily, chosen for this study is based on recent clinical trials in drug-susceptible TB and substantial routine clinical practice demonstrating that this dose is safe and well-tolerated [Boeree 2015]. Further, the EBA of 1200 mg of rifampin is measurable and higher than that of 600 mg in patients with drug-susceptible TB [Diacon 2007; Boeree 2015]. The tolerability of rifampin at doses higher than 20 mg/kg daily is not well-established. Clinical trials have also shown supraproportional increases in exposure with increases in rifampin dose, such that doubling the rifampin dose increases drug concentrations 3- to 4-fold. According to experiments performed in a validated in vitro pharmacodynamics system (hollow fiber) model, a rifampin AUC/MIC of 24 h*mg/L is the target that maximizes bacterial kill. The median steady-state AUC_{0-24} for rifampin at a dose of 600 mg is 26 h*mg/L (range 21-41) and it is 113 h*mg/L (range 78-162) for a dose of 1200 mg [Boeree 2015]. With a co-administered cell wall synthesis inhibitor, cell wall permeability may theoretically increase, resulting in even higher intracellular concentrations. It is also known that rifampin has a prolonged post-antibiotic effect. In the current study, if a rifampin dose of 10 mg/kg were selected but did not augment the activity of meropenem and amoxicillin/clavulanate against *M. tuberculosis* isolates with *rpoB* mutations and phenotypic resistance, then there would remain legitimate uncertainty as to whether the negative result was simply due to insufficient rifampin dose and exposure – an issue that could be overcome with relative ease. Even with a single dose level of rifampin, exposures will be highly variable, and the MIC range for isolates from patients with drug-susceptible and drug-resistant TB will range widely, allowing for robust PK-PD analyses. The dose of amoxicillin/clavulanate (500 mg/125 mg) three times daily has regulatory approval for other non-TB indications and is used widely in routine clinical practice with a good safety record; this dose in combination with meropenem has been shown to have substantial EBA against *M. tuberculosis* [Diacon 2016]. In this study amoxicillin/clavulanate is used mainly for the beta-lactamase inhibition conferred by clavulanate, which is not currently available as a single component drug for use in humans. However, the exposure of study participants to concomitant amoxicillin is justified by a) in vitro data showing that the addition of amoxicillin to meropenem/clavulanate reduces the minimum inhibitory concentration of meropenem substantially beyond that of clavulanate alone [Gonzalo 2013], and b) considerable clinical experience with the use of meropenem with amoxicillin/clavulanate for treatment of highly drug-resistant TB.

Additionally, this study will characterize the relationship between meropenem exposure (in combination with amoxicillin/clavulanate) and early bactericidal activity in order to identify the PK/PD parameter most closely linked to efficacy and target parameter value for the desired effect. In participants with drug-

susceptible TB, the combination of meropenem plus amoxicillin/clavulanate (without rifampin) will be administered intravenously at three different dosages: meropenem two grams thrice daily, meropenem one gram thrice daily, and meropenem three grams once daily. To identify the PK/PD parameter most closely linked to efficacy, the same three gram total daily dose will be administered as three grams once daily or as one gram thrice daily. The dose fractionation methodology will enable a quantitative analysis to determine which PK/PD parameter is most closely correlated with EBA. The dose-ranging thrice daily arms will enable a quantitative analysis to determine the target value of that parameter associated with bactericidal effect. These results will inform the identification of more feasible dosing strategies for meropenem (e.g. 3 grams once daily) and other marketed carbapenems for tuberculosis patients in the near term, as well as the dosage selection for novel oral carbapenems that may be available for tuberculosis treatment in the future. As described below, meropenem dosages of 2 grams thrice daily and 1 gram thrice daily are approved by the US FDA for certain indications. Once daily administration of meropenem 3 grams is investigational, and there is limited published information on use of this dosage for treatment of respiratory infections in adults [Etemadi 2011, Darley 2000].

3.4 Potential Risks and Benefits

There are several potential risks to study participants. There is a risk of clinically significant TB disease progression while on investigational study drugs. To reduce this risk, severely ill individuals will not be enrolled in this study, and in addition the investigational treatment is restricted to 14 days. There is a risk of toxicity of the investigational drugs. To reduce this risk, eligibility parameters are such that individuals having meaningfully abnormal results for key hematology and/or chemistry parameters will not be enrolled. In addition, participants will be monitored closely for adverse events. There are minimal medical risks associated with drawing blood and obtaining sputum.

There is no direct benefit to study participants. This study will benefit society by contributing to the understanding of optimal strategies for treatment of TB.

4 STUDY DRUGS

4.1 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.

Dose and indications: Rifampin is indicated in the treatment of all forms of tuberculosis [Rifadin package insert]. The recommended dose of rifampin for the treatment of TB in adults is 10 mg/kg in a single daily administration, not to exceed 600 mg/day. Higher doses of rifampin (900 mg daily to 1200 mg daily) have been used in other infections such as brucellosis, leishmaniasis, legionnaires' disease, and bone/joint infections without apparent safety or tolerability problems [Solera 1995; Kochar 2000; Edelstein 1984; Kissling 1981; Zimmerli 2004]. Higher doses of rifampin, up to 35 mg/kg daily, are under investigation for treatment of tuberculosis based on in vitro, animal, and clinical trial evidence that higher doses of rifampin may be more effective against *M. tuberculosis* [Gumbo 2007; Jayaram 2003; Rosenthal 2012;Jindani 1980; Jindani 2003; Diacon 2007; Ruslami 2007; Boeree 2015]. Rifampin is available in 150 mg or 300 mg capsules. In this study, rifampin will be provided at a dose of 20 mg/kg daily.

Pharmacokinetics: After 1.5 to 2 hours, a 600 mg dose yields a peak blood level of 6-10 mg/L, while a 1200 mg dose yields a C_{max} of 16-32 mg/L [Boeree 2015]. The steady-state AUC_{0-24} for rifampin at a dose of 600 mg is 26 h*mg/L (range 21-41) and it is 113 h*mg/L (range 78-162) for a dose of 1200 mg. Rifampin is normally absorbed completely when taken orally, but food delays absorption. 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.

Drug-drug interactions: Rifampin is an inducer of a number of hepatic enzymes involved in the metabolism of drugs and some hormones. This enzyme induction causes more rapid elimination (and potential loss of efficacy) of many drugs. Medications for which concomitant rifampin is contraindicated include: HIV-1 protease inhibitors (other than ritonavir), delavirdine, cyclosporine, tacrolimus, itraconazole, and ketoconazole. For many other medications, the dose can be increased to compensate for the effect of rifampin.

Toxicity and Tolerability: In the usual daily dose of 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 AEs 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 administered intermittently can cause a flu-like syndrome of fever, chills, and myalgia, 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. There may be changes in menstruation. For the treatment of tuberculosis, a rifampin dose of 20 mg/kg daily administered for 5 days was safe and well-tolerated (Diacon 2007). A recent dose-ranging trial showed that two weeks of rifampin

up to 35 mg/kg daily (the highest dose studied) was safe and well-tolerated (Boeree 2015); preliminary data from a study using a dose of 35 mg/kg daily for 12 weeks similarly showed that this dose was safe and well-tolerated (personal communication, M. Boeree).

4.2 Meropenem

Meropenem is a broad-spectrum carbapenem antibiotic that is active against Gram-positive and Gram-negative bacteria. Meropenem readily penetrates the cell wall of most Gram-positive and Gram-negative bacteria and inhibits cell wall synthesis through inhibition of both classical D, D-transpeptidases as well as recently discovered non-classical L, D transpeptidases including those of *M. tuberculosis*.

Dose and indications: Meropenem is indicated for treatment of complicated skin and skin structure infections caused by susceptible bacteria, treatment of complicated appendicitis and peritonitis caused by susceptible bacteria, and meningitis caused by susceptible bacteria [Merrem package insert]. The recommended dose of meropenem, administered intravenously, is 500 mg given every 8 hours for skin and skin structure infections, 1 gram every 8 hours for intra-abdominal infections, and up to 2 grams every 8 hours for meningitis [Merrem package insert]. Meropenem administered at a dosage of 3 grams once daily has been used in a limited number of adults for the treatment of respiratory infections. Darley and colleagues administered meropenem3 grams as a single intravenous dose once every 24 hours, for up to 10 days, to nine ambulatory adults (4 males and 5 females, average age 60 years) with infective exacerbations of bronchiectasis; 8 of 9 patients were effectively treated, and the meropenem is described as being well-tolerated although no safety information is reported [Darley 2000]. Etemadi and colleagues described the use of a once daily intravenous infusion of 3 grams of meropenem for treatment of aspiration pneumonia in 31 elderly patients (17 males, 14 females, average age 85 years) in long-term care facilities; this treatment was described as being clinically effective and tolerable, although no safety details are provided [Etemadi 2011]. In the here-in proposed trial, meropenem will be administered by intravenous infusion at the approved dosages of 2 grams every 8 hours and 1 gram every eight hours; in addition meropenem will be administered at the investigational dose of 3 grams once every 24 hours.

Pharmacokinetics: Meropenem is administered intravenously as an infusion or bolus injection. Meropenem rapidly and substantially penetrates most tissues including pulmonary tissues (Byl 1999). At the end of a 30-minute intravenous infusion of a single dose of meropenem in healthy volunteers, mean peak plasma concentrations are approximately 23 ug/mL (range 14-26) for a 500 mg dose, and 49 ug/mL (range 39-58) for a 1 gram dose. A 5-minute intravenous bolus injection in healthy volunteers results in mean peak plasma concentrations of 45 ug/mL (range 18-65) for a 500 mg dose and 112 ug/mL (range 83-140) for a 1 gram dose. In adults with normal renal function, the elimination half-life of meropenem is approximately one hour. Approximately 70% of the intravenously administered dose is recovered as unchanged meropenem in the urine over 12 hours. Plasma protein binding of meropenem is approximately 2%. There is one metabolite that is microbiologically inactive. In patients with renal insufficiency the plasma clearance of meropenem correlates with creatinine clearance. There is no detectable effect of liver disease on the pharmacokinetics of meropenem.

Drug-drug interactions: Probenecid competes with meropenem for active tubular secretion and inhibits the renal excretion of meropenem, leading to substantial increases in the elimination half-life of meropenem and in the extent of systemic exposure. Meropenem may reduce serum levels of valproic acid to subtherapeutic levels. Unlike many other candidate antimycobacterial compounds, meropenem has no interactions with rifampin.

Toxicity and Tolerability: Meropenem is contraindicated in patients with known hypersensensitivity to other carbapenems and in patients who have demonstrated anaphylactic reactions to beta-lactams. Seizures

and other adverse central nervous system experiences have been reported during meropenem treatment, but are rare and have occurred mostly in patients with pre-existing central nervous system disorders or with bacterial meningitis and/or renal insufficiency. During clinical investigations 2904 immunocompetent adults were treated with meropenem for infections outside the central nervous system – seizures occurred in 20 (0.7%) of patients, all of whom had pre-existing contributing factors including prior history of seizures, or central nervous system abnormality and concomitant medications with seizure potential. During clinical investigations of 2904 immunocompetent adults the following adverse reactions were reported, irrespective of the relationship to therapy with meropenem, in greater than 1.0% of patients: diarrhea (4.8%), nausea/vomiting (3.6%), inflammation at the injection site (2.4%), headache (2.3%), rash (1.9%), sepsis (1.6%), constipation (1.4%), apnea (1.3%), shock (1.2%), and pruritis (1.2%). Pseudomembranous colitis due to Clostridium difficile has been reported with nearly all antibacterial agents, including meropenem.

4.3 Amoxicillin/clavulanate

Amoxicillin/clavulanate (abbreviated as Amx/Clv) is an oral antibacterial combination consisting of amoxicillin and clavulanate potassium. Amoxicillin is a semisynthetic analog of ampicillin. Amoxicillin is susceptible to degradation by beta-lactamases and therefore its clinical spectrum of activity does not include organisms which produce these enzymes. Clavulanate is a beta-lactam that inactivates a wide variety of beta-lactamases by blocking the active sites of these enzymes. Thus, the formulation of amoxicillin and clavulanate protects amoxicillin from degradation by beta-lactamases and effectively extends the antibiotic spectrum of amoxicillin.

Dose and indications: Amx/Clv is indicated for treatment of lower respiratory tract infections, otitis media, and sinusitis caused by beta-lactamase-producing strains of Haemophilus influenza and Moraxella catarrhalis, as well as skin and skin structure infections caused by beta-lactamase-producing strains of Staphylococcus aureus, Escherichia coli, and Klebsiellaspp; and urinary tract infections caused by beta-lactamase-producing strains of E. coli, Klebsiella spp., and Enterobacterspp [Augmentin package insert]. In adults, the recommended dose of Amx/Clv for treatment of severe infections and infections of the respiratory tract is one 500 mg tablet (500 mg amoxicillin/125 mg clavulanate) every 8 hours or one 875 mg tablet (875 mg amoxicillin/125 mg clavulanate) every 12 hours. For this clinical trial, Amx/Clv will be administered at a dose of one 500 mg tablet every 8 hours or one 875 mg tablet once daily, depending on treatment assignment.

Pharmacokinetics: Amoxicillin and clavulanate potassium each are well-absorbed from the gastrointestinal tract after oral administration. While the absorption of clavulanate potassium is greater when taken with food relative to the fasted state, the safety and efficacy of the Amx/Clv combination has been established in clinical trials where it was taken without regard to food. Amoxicillin serum concentrations achieved with Amx/Clv are similar to those produced by oral administration of equivalent doses of amoxicillin alone. After oral administration of Amx/Clv the elimination half-life of amoxicillin is 1.3 hours and that of clavulanate potassium is 1.0 hour. Approximately 50-70% of amoxicillin and approximately 25-40% of the clavulanate potassium are excreted unchanged in the urine during the first 6 hours after administration. Serum protein binding has been estimated at approximately 18% for amoxicillin and approximately 25% for clavulanate potassium. Amoxicillin and clavulanate each diffuse readily into most tissues except brain and spinal fluid.

Drug-drug interactions: Probenecid decreases the renal tubular secretion of amoxicillin and can result in increased and prolonged blood levels of amoxicillin. Amx/Clv may reduce the efficacy of oral contraceptives. Amx/Clv has no clinically meaningful drug-drug interactions with rifampin.

Toxicity and Tolerability: Amx/Clv is contraindicated in patients with a history of allergic reactions to any penicillin and in patients with a previous history of cholestatic jaundice/hepatic dysfunction associated with Amx/Clv. Pseudomembranous colitis due to Clostridium difficile has been reported with nearly all antibacterial agents, including Amx/Clv. Hepatic dysfunction, including severe cholestatic hepatitis, has rarely been reported with Amx/Clv. Amx/Clv is generally well-tolerated. The majority of side effects observed in clinical trials of Amx/Clv were mild and transient; less than 3% of patients discontinued therapy because of drug-related side effects. In the original premarketing studies that included both adults and children, the most frequently reported adverse effects were diarrhea/loose stools (9%), nausea (3%), skin rashes and urticarial (3%), vomiting (1%), and vaginitis (1%). Serum sickness-like reactions, erythema multiforme, exfoliative dermatitis, hepatitis, interstitial nephritis, and convulsions each have been reported with ampicillin-class antibiotics but are rare.

5 DESIGN

This will be a prospective, randomized, open label phase 2a clinical trial. This study will enroll two groups of participants, namely participants with the presence of rifampin resistance-conferring *rpoB* mutations in *M. tuberculosis*(Group 1) and participants without the presence of rifampin resistance-conferring *rpoB* mutations in *M. tuberculosis*(Group 2). Within Group 1, participants will be randomized to receive one of the two treatment regimens. Within Group 2, participants will be randomized to receive one of the four treatment regimens.

5.1 Treatment Regimens

Thetreatment regimens are shown below. Regimens A and C are comprised of meropenem plus amoxicillin/clavulanate with rifampin. Regimens B, D, E, and F are comprised of meropenem plus amoxicillin/clavulanate without rifampin.

In study arms A, B, C, and D, meropenem will be administered intravenously at a dosage of 2 grams thrice daily. In arms E and F, a total of 3 grams of meropenem will be administered each day; in arm E the meropenem will be administered in divided doses (1 gram thrice daily), and in arm F the meropenem will be administered as a single once daily dose of 3 grams.

Rifampin will be administered orally at a dose of 20 mg/kg once daily (arms A and C). Amx/Clv will be administered orally at a dose of 500 mg/125 mg thrice daily, except in arm F in which Amx/Clv will be administered orally at a dose of 875 mg/125 mg once daily.

Study treatment will be administered for 14 days. Additional details are in Section 8.1.

	Regimen	**Number per arm**	**Regimen components**		
Rifampin resistant	A	24	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	B	16	-	Meropenem 2 grams thrice daily	Amx/Clv
Rifampin susceptible	C	12	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	D	12	-	Meropenem 2 grams thrice daily	Amx/Clv
	E	12	-	Meropenem *1 gram thrice daily*	Amx/Clv
	F	12	-	Meropenem *3 grams once daily*	Amx/Clv

Abbreviation: Amx/Clv, amoxicillin/clavulanate

5.2 Endpoints

5.2.1 Primary Endpoints

- Mean daily fall in $\log_{10}$CFU of *M. tuberculosis* per mL sputum over14 days of treatment ($EBA_{0\text{-}14}$(CFU)

- Steady state PK and PK/PD parameters (including $AUC_{0\text{-}24}$, maximum concentration (C_{max}), MIC against *M. tuberculosis*; time above MIC) for rifampin and meropenem

5.2.2 Secondary Endpoints

- Grade 2 or higher drug-related adverse clinical or laboratory events
- Change in time-to-positivity in MGIT liquid media over 14 days of treatment ($EBA_{0\text{-}14}$(TTP))
- Mean daily fall in log_{10}CFU of *M. tuberculosis* per mL sputum over2 days of treatment ($EBA_{0\text{-}2}$(CFU)

5.3 Randomization

Participants will be assigned to the 'Rifampin Resistant' group (Group 1) or the 'Rifampin Susceptible' group (Group 2) based on the presence or absence, respectively, of a rifampin resistance-conferring mutation identified using Xpert MTB/RIF rapid testing at study screening. Participants in the Rifampin Resistant group will be randomized 3:2 to study arms A and B. Participants in the Rifampin Susceptible group will be randomized 1:1:1:1 to study arms C, D, E, and F. Randomization will be computer-generated.

Once accrual into a group (i.e. the Rifampin Resistant group or the Rifampin Susceptible group) is completed, enrollment into that group will be closed and participants who are found eligible for that group based on Xpert MTB/RIF rifampin DST results will not be randomized into the trial.

This will be an open-label trial in which neither the subjects nor the study staff will be blinded as to rifampin resistance/susceptibility group status or treatment assignment after randomization. However, microbiologists performing mycobacterial EBA cultures will be blinded both to rifampin resistance/susceptibility group status and treatment assignment after randomization. Statisticians will be blinded as to treatment assignment of individual patients.

6. POPULATION

6.1 General Considerations

Study entry is open to males and females age 18 years to 65 years of any ethnic background who meet study eligibility criteria. Historically, approximately 60% of patients with TB at clinical sites are males. The gender, ethnicity, and socioeconomic background of study subjects are expected to mirror that of the population served by the study site, and that of the population most affected by TB. Study subjects will be recruited from clinics in Cape Town, South Africa.

6.2 Special Populations

6.2.1 Children, Pregnant Women, and Breast-Feeding Women

Children will not be enrolled in this exploratory EBA study because of differences in the typical disease presentation in children versus adults; study participants must be able to produce a 16-hour sputum specimen daily for seven days and few children can do this. Pregnant women or breast-feeding women will not be enrolled since a 14-day delay in initiation of definitive TB treatment could increase the risk of TB transmission to the fetus/newborn. In addition, pregnancy may affect study drug pharmacokinetics and introduce a source of variability that cannot be adequately addressed in this small PK and safety study.

6.2.2 Prisoners

This study will not enroll prisoners.

6.2.3 HIV-Positive Individuals

HIV-infected individuals with CD4 ≥100 cells/cu mm as well as HIV-uninfected individuals will be included in this study. HIV is a risk factor for TB and drug-resistant TB, and therefore the identification of improved TB treatment strategies that can be used in people living with HIV/AIDS is important. HIV-infected adults with severe immunosuppression (for this study defined as CD4 < 100 cells/cu mm) and concomitant TB have high early mortality rates, and therefore prompt definitive therapy for both TB and HIV is warranted; such individuals will not be eligible for study participation. Rifampin has clinically significant drug-drug interactions with some antiretroviral drugs, and thus HIV-infected individuals receiving treatment with certain antiretroviral drugs will not be eligible for study participation.

6.3 Eligibility Criteria

6.3.1 Inclusion criteria for participation in this study

- New or recurrent pulmonary TB with one or both of the following:
    - sputum positive for acid-fast bacilli on direct microscopy of at least grade 1+ (International Union Against Tuberculosis and Lung Disease [IUATLD] scale) on at least one pre-treatment sputum sample
    - sputum positive for *M. tuberculosis* by Xpert MTB/RIF testing, with semiquantitative result of ‘medium’ or ‘high’ on at least one pre-treatment sputum sample
- Age ≥18 and ≤65 years at study screening
- Ability and willingness to provide informed consent
- Body weight 40 kg to 90 kg, inclusive

- Laboratory values obtained within 30 days prior to or at study screening:
  - Absolute neutrophil count (ANC) ≥750 cells/mm^3
  - Hemoglobin ≥7.0 g/dL
  - Platelet count ≥50,000/mm^3
  - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤3 X upper limit of normal (ULN)
  - Total bilirubin ≤2.5 X ULN
  - Creatinine< 1.5 X ULN

- HIV infection must be documented as either absent or present

- For HIV-positive candidates only: CD4+ cell count of ≥ 100 cells/cu mm, performed within 30 days prior to or at study screening

- For females of reproductive potential, negative serum or urine pregnancy test within 7 days prior to or at study screening. Female participants who are participating in sexual activity that could lead to pregnancy must agree to use one reliable non-hormonal method of contraception (condoms or an IUD), or another method (diaphragm or cervical cap) if it is approved by the national regulatory authority and used according to package insert, while receiving study medications.

- Willingness to be hospitalized for a minimum of 16 consecutive days

- Ability to produce an overnight sputum sample of sufficient quality and quantity. As a guideline, this should be10 ml or more during a 16-hour collection period. Volume is clinically estimated from a spot sample provided at screening and verified upon the first overnight collection (which can be repeated upon retraining).

- Xpert MTB/RIF result performed on sputum within 14 days prior to or at study screening that shows EITHER 'Rifampin resistance detected' OR 'Rifampin resistance not detected'.

6.3.2 Exclusion Criteria

- Treatment with any drug active against *M. tuberculosis* within the 3 months prior to study screening.

- Breast-feeding

- Known allergy or sensitivity to any of the study drugs

- Participants receiving valproate sodium or probenecid

- Karnofsky score < 60 OR poor general condition such that, in the opinion of the investigator at screening, any delay in initiation of definitive TB treatment cannot be tolerated

- Known current neurological TB or seizure disorder

- Any condition as determined by physical examination, medical history, laboratory data, or chest x-ray which, in the opinion of the investigator, would interfere with safety or endpoint assessments in the study.

7. STUDY SCHEDULE

Appendix A contains a table of the Schedule of Events.

7.1 Screening Visit

Subjects with a presumptive diagnosis of sputum smear positive pulmonary TB will be invited to participate.

Informed written consent, using IRB-approved consent forms, will be obtained by trained study personnel prior to performing any study-specific procedures. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. The informed consent process is described in detail in the "Study Procedures" section.

The screening period will be kept as short as possible, with a maximum duration of 7 days. Enrollment sputum collections can be started before all screening results are available.

The following will be performed after obtaining written informed consent:

- Participants will be asked questions related to demographics.
- Medical history
- Medication history
- Assessment of signs, symptoms, and diagnoses
- Complete physical examination
- Height
- Weight
- Karnofsky score
- Complete blood count with differential
- Complete blood chemistry profile
- Pregnancy test (women)
- CD4+ count (HIV-infected participants)
- Chest X-ray (unless results of a chest X-ray performed within 14 days prior to screening are available)
- Spot sputum collection for AFB smear microscopy and Xpert MTB/RIF
- Counseling about study procedures
- Hospitalisation (at the discretion of the investigator)

7.2 Enrollment (Day -1)

Procedures to be performed at Enrollment:

- Concomitant medication assessment / review of eligibility criteria with respect to medication use
- Enrollment of individuals who meet eligibility criteria
- Assignment to Group 1 (rifampin resistant) or Group 2 (rifampin susceptible)*
- Randomization*
- Hospitalization
- Spot sputum collection for culture, with cultivated *M. tuberculosis* then tested using a commercially available rapid molecular test for resistance/susceptibility to isoniazid, rifampin, fluoroquinolones, and second-line injectable drugs; b) phenotypic DST; c) MIC determination
- Overnight sputum collection
- Counseling about study procedures

Participants who do not meet eligibility criteria or are not enrolled will be referred to a local source of TB care. *Group assignment and randomization may be performed either on Day -1 or on Day 0, depending on when rifampin resistance results are available.

7.3 Day 0

The participant will remain hospitalized. Procedures to be performed on Day 0:

- Group assignment and randomization, if not already performed on Day -1
- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Concomitant medication assessment
- Pregnancy test (women)
- Overnight sputum collection
- Counseling about study procedures
- Blood and urine retention sample collection

7.4 Study Days 1, 2, 3, and 4

The participant will remain hospitalized and will receive assigned study treatment starting on Day 1. The following procedures will be performed on each of days 1, 2, 3, and 4:

- Administration of assigned study treatment
- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Overnight sputum collection
- Counseling about study procedures
- IN ADDITION, blood and urine retention sample collection on day 3

7.5 Study Days 5, 6, 7, 8, 9, 10, 11, and 12

The participant will remain hospitalized and will receive assigned study treatment. The following procedures will be performed on each day:

- Administration of assigned study treatment
- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Counseling about study procedures
- IN ADDITION, overnight sputum collection will occur on days 6, 8, 10, and 12
- IN ADDITION, a complete blood count with differential and a complete chemistry profile will be performed on day 7
- IN ADDITION, blood and urine retention sample collection on day 7

7.6 Study Day 13

The participant will remain hospitalized. The following procedures will be performed:

- Administration of assigned study treatment
- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Counseling about study procedures
- Pharmacokinetic sampling

7.7 Study Day 14

The participant will remain hospitalized. The following procedures will be performed:

- Administration of assigned study treatment
- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Pregnancy test (women)
- Complete blood count with differential
- Complete blood chemistry profile
- Overnight sputum collection
- Counseling about study procedures
- Blood and urine retention sample collection

7.8 Study Day 15

The following procedures will be performed:

- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- The participant will be discharged from the hospital

7.9 Study Day 28 (Visit window +/- 3 days)

The following procedures will be performed:

- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Complete blood count with differential
- Complete blood chemistry profile

7.10 Early withdrawal

In case of early withdrawal during the study treatment period the following information will be collected and procedures performed:

- Targeted physical examination
- Weight
- Assessment of signs, symptoms, and diagnoses
- Adverse events assessment
- Concomitant medication assessment
- Complete blood count with differential
- Complete blood chemistry profile
- Blood and urine retention sample collection

8. STUDY TREATMENT

8.1 Regimens, Administration, and Duration

The treatment regimens are shown below. Regimens A and C are comprised of meropenem plus amoxicillin/clavulanate with rifampin. Regimens B, D, E, and F are comprised of meropenem plus amoxicillin/clavulanate without rifampin.

In study arms A, B, C, and D, meropenem will be administered intravenously at a dosage of 2 grams thrice daily, with infusion duration approximately 30 minutes. In arms E and F, a total of 3 grams of meropenem will be administered each day; in arm E the meropenem will be administered in divided doses (1 gram thrice daily, infusion duration approximately 30 minutes for each dose), and in arm F the meropenem will be administered as a single once daily dose of 3 grams (infusion duration approximately 60 minutes for each dose).

Rifampin will be administered orally at a dose of 20 mg/kg once daily (arms A and C only).
Amx/Clv will be administered orally at a dose of 500 mg/125 mg thrice daily in arms A, B, C, D, and E.
In arm F, Amx/Clv will be administered orally at a dose of 875 mg/125 mg once daily.
Oral study drugs will be administered at the time that the meropenem infusion starts.

Study treatment will be administered for 14 days.

	Regimen	**Number per arm**	**Regimen components**		
Rifampin resistant	A	24	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	B	16	-	Meropenem 2 grams thrice daily	Amx/Clv
Rifampin susceptible	C	12	Rifampin	Meropenem 2 grams thrice daily	Amx/Clv
	D	12	-	Meropenem 2 grams thrice daily	Amx/Clv
	E	12	-	Meropenem *1 gram thrice daily*	Amx/Clv
	F	12	-	Meropenem *3 grams once daily*	Amx/Clv

Abbreviation: Amx/Clv, amoxicillin/clavulanate

8.2 Study Product Formulation and Preparation

Rifampin will be supplied as Rifadin® (rifampin capsules USP) by Sanofi-Aventis.

Meropenem will be supplied as Merrem® I.V. (meropenem for injection), 1 gram infusion vials, by AstraZeneca. Infusion vials will be constituted with sodium chloride 0.9%. Details of constitution are provided in the Manual of Procedures.

Amoxicillin/clavulanate will be supplied by GlaxoSmithKline as Augmentin® 500 mg tablets (containing 500 mg amoxicillin and 125 mg clavulanate) for Arms A, B, C, D, and E and as Augmentin® 875 mg tablets (containing 875 mg amoxicillin and 125 mg clavulanate) for Arm F.

8.3 Study Product Accountability

The site pharmacist will maintain records of all study products received, dispensed to study participants, and final disposition of all study products. These records will include the lot number and expiration date as well as the start and stop dates for usage of that lot number.

8.4 Concomitant Medications

The use of all non-study drugs including over-the-counter products from 14 days before study screening to completion of the final study visit will be monitored and recorded.

8.4.1 Prohibited Medications

Use of anti-tuberculosis medications (other than assigned study treatment) is prohibited from screening until after the final dose of study medications. A list of prohibited anti-tuberculosis medications is provided in the Manual of Procedures.

Use of the following drugs is prohibited from screening until after the final dose of study medications: probenecid, chloramphenicol, macrolide antibiotics, sulfonamides, tetracycline antibiotics, tramadol, ketoconazole, antacids.

Antiretroviral drugs are prohibited except for efavirenz, nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors, which are allowed if, at the time of study screening, the participant has already been started on those drugs and is stable on them. Antiretroviral drugs including efavirenz, nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors should not be newly initiated between screening and the final dose of study medications.

8.5 Adherence Assessment

Study doses will be observed during hospitalization and adherence will be documented by study staff.

9. STUDY PROCEDURES

9.1 Informed Consent Process

Informed written consent, using IRB-approved consent forms, will be obtained by trained study personnel prior to performing study-specific procedures. Potential subjects will receive information about risks and possible benefits of study participation, study objectives and procedures, potential toxicities, and the informed consent process. Informed consent requires the signature or mark of the subject. A copy of the signed and dated informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in this study, and that they may withdraw consent at any time. Individuals who choose not to participate in the study will be referred to local sources of TB care.

9.2 Clinical and Laboratory Evaluations

9.2.1 Instructions for Clinical Evaluations

Medical History
The medical history should include clinical diagnoses and events, as well as allergies to medications.

Medication History
TB treatment should be recorded on the CRF.

The following medications, if administered/taken within 14 days prior to study screening, should be recorded on the study CRF: antiretroviral medications, prescription drugs for treatment of opportunistic infections, prescription drugs for prophylaxis of opportunistic infections, other prescription drugs, alternative therapies, dietary supplements.

Complete Physical Examination
A complete physical examination will include at a minimum an examination of the skin, head, mouth, neck and lymph nodes; auscultation of the chest; cardiac exam; abdominal exam; examination of the lower extremities for edema. The complete physical exam will also include signs and symptoms, diagnoses, and vital signs (temperature, pulse, respiration rate, and blood pressure).

Targeted Physical Examination
A targeted physical examination will include vital signs (temperature, pulse, respiration rate, and blood pressure). The targeted physical examination is to be driven by any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit.

Karnofsky Score
Karnofsky score will be recorded at screening.

Height
Height will be recorded once at screening.

Weight
Weight will be recorded at all study visits except as noted in the Schedule of Events.

Signs and Symptoms
At screening all grades of signs and symptoms that occurred within 7 days prior to screening must be recorded. Post-screening, only signs and symptoms of Grade 2 or higher must be recorded. In addition, all signs and symptoms that lead to a change in study treatment must be recorded, regardless of grade.

Diagnoses
Record all diagnoses identified for clinical events and other diseases.

Concomitant Medications
All concomitant medications started or stopped since the last study visit must be recorded on the CRF.

TB Medications
Except for assigned study medications, TB treatment should not be administered on Days 0 through the completion of the overnight sputum collection on Day 15. After completion of the overnight sputum collection on Day 15, and no later than Day 18, the participant should be started on TB treatment according to local norms. The start date and initial doses should be recorded.

Antiretroviral Medications
Record all modifications, including start/stop dates, initial doses, participant-initiated and/or health care provider-mandated modifications, and any temporary holds or permanent discontinuations.

9.2.2 Instructions for Laboratory Evaluations

Complete Blood Count with Differential
Complete all values regardless of grade for hemoglobin, platelet count, white blood cell count, absolute neutrophil count.

Complete Blood Chemistry Profile
Record all values regardless of grade for AST, ALT, alkaline phosphatase, total bilirubin, creatinine, potassium, and albumin.

CD4+ Count (HIV-infected participants)
For participants who are HIV-infected, a CD4+ cell count determination performed within 30 days prior to or at study screening must be recorded.

Pregnancy Test
For women with reproductive potential: serum or urine beta-HCG. A urine test, if used, must have a sensitivity of 15-25 mIU/mL.

Chest X-ray
A chest X-ray will be performed at screening, unless the results of a chest X-ray performed within 14 days prior to screening are available.

9.2.3 Instructions for Sputum Samples

Details of laboratory procedures will be described in the Manual of Operations.

Spot Sputum

This will be collected under supervision at the study site. A sample as close to 5 mL should be produced if possible.

Overnight Sputum Collection

Sputum will be collected over approximately 16 hours each night beginning at around 4 pm +/- one hour in an appropriate clean container in hospitalized individuals. This sputum will be used for EBA analysis. Start and stop times, sputum volume collected and sputum quality will be recorded.

Solid Culture CFU Determination and Liquid MGIT Culture TTP

For the determination of drug activity, sputum will be cultured on solid medium and CFU counts will be determined. Sputum will also be cultured in MGIT liquid medium using an automated detection system; daily TTP, measured in hours, will be performed.

Other Mycobacteriology Testing

Cultured *M. tuberculosis* from the Enrollment Day spot sputum or the first positive sputum culture will be subjected to drug susceptibility testing using the following methods: a) commercially available rapid molecular test that includes testing for isoniazid, rifampin, fluoroquinolones, and second-line injectable agents (e.g. Hain Genotype MTBDRplus and Genotype MTBDRsl); b) phenotypic rifampin susceptibility test using MGIT culture; and c) determination of MICs to study drugs. DNA sequencing of the *M. tuberculosis* rpoB gene may be performed in order to correlate rpoB mutations with rifampin MICs.

9.2.4 Pharmacokinetic Sampling

Details of sampling procedures will be provided in the Manual of Operations. Blood samples for study drug concentrations may be collected from an indwelling catheter, or, if a catheter cannot be placed or maintained successfully, by direct venipuncture.

9.2.5 Blood and urine retention sampling

The blood and urine retention sampling methodology and requirements will be described in a separate document, the Biomarker Manual, which will be provided prior to the trial start. These samples will be collected in addition to the trial safety laboratory evaluations on days 0, 3, 7, and 14 and Early Withdrawal. The samples will be stored for potential future analysis of biomarkers related to tuberculosis and its treatment (e.g. biomarkers as novel surrogate markers of efficacy). These samples will not be used for genetic testing.

9.2.6 Hospitalization

At the discretion of the investigator, a participant may be hospitalized during the screening period if necessary for the safety of the participant or to decrease risk of tuberculosis transmission to others. All participants who receive study treatment will be hospitalized from Day -1 to Day 15. The period from screening to treatment will be kept as short as logistically possible to minimize the period of participants remaining without TB treatment.

9.3 Compensation of Study Subjects

Study subjects will be compensated for their time and travel to and from study visits as well as for visits and for time spent waiting for results while in hospital. The form and amount of compensation will be in accordance with local guidelines.

10. ASSESSMENT OF SAFETY AND TOLERABILITY

10.1 Safety and Tolerability Endpoints

The primary endpoint for analysis of safety and tolerability is Grade 2 or higher adverse events.

10.2 Safety Evaluation

A Data and Safety Monitoring Board (DSMB) will review the study protocol and oversee progress of the trial. The DSMB will review safety data once 50% of the participants have completed study treatment. Since this is an open-label study, the DSMB will have access to treatment group assignment. The DSMB will be comprised of at least the following: an expert in statistics, an expert in clinical trials, and an expert in clinical TB. No early stopping rules will be formally adopted.

No interim efficacy analyses are planned for this Phase 2 trial.

10.3 Study Treatment Discontinuation Criteria

Participants who prematurely discontinue study treatment after having received one or more doses of assigned treatment will be replaced.

Study treatment must be immediately discontinued as a result of the following:

- Withdrawal of informed consent.
- Investigator considers it for safety reasons in the best interest of the participant that study treatment be stopped.
- Participant becomes pregnant.
- Termination of the study by the sponsor.

All participants withdrawn from treatment will be referred to the local community TB clinics for standard antituberculosis chemotherapy according to National Guidelines. The participants will be provided with a referral letter to take with them to the TB Clinic. Participants will be transported by the study staff to the clinic, or a follow-up call will be made by the study site staff to the clinic to determine if the participant attended the clinic on the date as arranged. Every effort will be made to continue to follow participants who discontinue study medicine prematurely until the final study visit, if they are agreeable.

10.4 Adverse Events

The Investigators are responsible for eliciting adverse events by observing study participants and recording all adverse events observed by him/her or reported by study participants during the trial.

10.4.1 Definitions

Adverse event (AE): Any untoward medical occurrence in a study participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Serious adverse event (SAE): Any untoward medical occurrence that at any dose:

- results in death;
- is life threatening (any event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- is a medically important event.

  Note:
  Medical and scientific judgment should be exercised in deciding events are "medically important." These events are those that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the study participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Unexpected Adverse Event (UAE): An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

10.4.2 Association and Attribution/causality

An adverse event is considered associated with the use of the drug (Adverse Drug Reaction) if the attribution is possible, probable or very likely.

The following definitions for rating attribution/causality will be:

Relatedness Rating	**Definition**
Not Related	An adverse event, which is not related to the use of the drug.
Doubtful	An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s) or concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.
Possible	An adverse event, which might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s) or concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be excluded.

Probable	An adverse event, which might be due to the use of the drug. The relationship in time is suggestive, e.g., confirmed by dechallenge. An alternative explanation is less likely, e.g., concomitant drug(s) or concomitant disease(s).
Definite	An adverse event, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s) or concomitant disease(s).

10.4.3 Severity

All symptoms and laboratory findings will be graded according to severity using the Division of AIDS Table for Grading Severity of Adult Adverse Events, which is a comprehensive grading scale for patients with infectious diseases, including TB or HIV.

10.4.4 Reporting

***AE**:* Adverse events will be collected by the investigators from the time a study participant receives the first dose of study drug through the final study visit. Any AE (serious or non-serious) observed by the investigator or reported by the study participant that meets the AE reporting requirements (see below) will be recorded on an Adverse Event Case Report Form. The Investigator will review each AE and assess its relationship to drug treatment based on all available information at the time of the completion of the case report form. The following information will be recorded for each Adverse Event reported:

- Diagnosis of the AE, if possible. In the case where an overall diagnosis cannot be made, each specific sign and/or symptom will be recorded as individual AEs;
- Date of onset;
- Stop Date (duration) if applicable;
- Severity;
- Action taken with study drugs;
- Other action taken;
- Outcome;
- Relationship to study drugs;
- Seriousness.

The following adverse events must be reported on an Adverse Event Report Form:

- new medical diagnosis (at the time of enrollment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported)
- any grade 2or higher adverse event
- study drug discontinuation due to an adverse event
- pregnancy

***SAE**:* Any SAE must be recorded and reported by the site investigator to the Johns Hopkins University study coordinator within 72 hours and to the local IRB and to regulatory authorities in accordance with local requirements and ICH guidelines for Good Clinical Practice. SAEs will be collected from the time a study participant receives the first dose of study drug through the final study visit.

The study drugs are rifampin, meropenem, and amoxicillin/clavulanate. The most common adverse effects associated with the study drugs are specified in the "Study Drugs" section. The investigator is responsible for monitoring all adverse events that are observed or reported during the study, regardless of whether they are related to study drugs.

In accordance with the FDA's Code of Federal Regulations, the study sponsor and the participating investigators are responsible for reviewing all information relevant to the safety of the study drugs. Reporting and monitoring of SAEs is required to alert the FDA, sponsor, institutional review boards, and the clinical investigators of real and potential safety issues. The investigators will carefully review the Adverse Event Reports and use this information to monitor the investigational drug's toxicity profile and patient safety. Any adverse experience associated with the use of the drug that is both serious and unexpected will be reported to the FDA in the form of a written Safety Report.

The responsible institutional review boards, FDA and South African Regulatory body (Medicines Control Council) will be notified of all SAEs and AEs according to their individual guidelines.

10.4.5 Pregnancy

For study purposes, pregnancy must be recorded on an Adverse Event form.

10.5 Clinical Management of Adverse Events

For all toxicities that are treatment-emergent and that require the study drugs 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. Toxicities of Grade 2 or higher and occurring during study treatment will be documented on the Adverse Event Form. The maximum level of toxicity reached will be clearly indicated.

10.5.1 Grade 1 toxicities

In general, for grade 1 toxicities, the patient will be followed carefully, and the study drugs will be continued.

10.5.2 Grade 2 toxicities

For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs temporarily held at the investigator's discretion.

10.5.3 Grade 3 toxicities

Unless the site investigator has compelling evidence that the Grade 3 AE or toxicity is not related to the study drug, the participant should permanently discontinue the study drugs.

10.5.4 Grade 4 toxicities:

Any patient with grade 4 renal, hepatic, cardiac or hematological toxicity will be immediately discontinued from study drugs.

11. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Details of the analyses specified in this section will be contained in a statistical analysis plan.

11.1 Hypotheses

- The 14-day antimycobacterial activity of rifampin added to meropenem plus amoxicillin/clavulanate will be higher than the 14-day antimycobacterial activity of meropenem plus amoxicillin/clavulanate alone, in patients with TB isolates with rifampin resistance-conferring *rpoB* mutations (A vs B) and also in patients with TB isolates without rifampin resistance-conferring *rpoB* mutations (C vs D).

- The effect of adding rifampin to meropenem plus amoxicillin/clavulanate, as measured by 14-day antimycobacterial activity, will be higher among patients with drug-sensitive TB than among patients with drug-resistant TB (A vs C).

- The effect of 1g meropenem plus amoxicillin/clavulanate, as measured by 14-day antimycobacterial activity, will be lower than that of 2g meropenem plus amoxicillin/clavulanate (D vs E).

- The effect of 3g meropenem plus amoxicillin/clavulanate once daily, as measured by 14-day antimycobacterial activity, will be lower than that of 2g meropenem plus amoxicillin/clavulanate three times a day (D vs F).

- An AUC/MIC for rifampin that achieves maximal effect when rifampin is added to meropenem plus amoxicillin/clavulanate can be determined by PK/PD modeling of combined PK and outcomes data from patients with infecting strains of *M. tuberculosis* that have resistance-conferring *rpoB* mutations and patients with infecting strains of *M. tuberculosis* that do not have resistance-conferring *rpoB* mutations

- The combination of meropenem, amoxicillin, clavulanate, and rifampin will be safe and well-tolerated

11.2 Data Analysis

Detailed data analysis plans are available in the Statistical Analysis Plan.

Analyses for primary endpoints:

- The 14-day EBA, or $EBA_{0\text{-}14}CFU$, as determined by the rate of change in $\log_{10}$ CFU per mL sputum over the period of Day 0 to Day 14 will be described with at most three parameters from a linear, bi-linear, or non-linear regression of $\log_{10}$ CFU on time. Descriptive statistics of $EBA_{0\text{-}14}CFU$ for each arm will be reported in a table. Box plots and a plot with confidence intervals will be created.

- Equivalence tests will be conducted to examine whether the $EBA_{0\text{-}14}CFU$ of Arm A is different from the $EBA_{0\text{-}14}CFU$ of Arm B. A similar comparison will be done comparing Arm C to Arm D, Arm A to Arm C, Arm D to Arm E, Arm D to Arm F, and Arm E to Arm F.

- The relationship between the AUC/MIC for rifampin (value of zero in Arms B and D; experimentally determined in Arms A and C) and the EBA will be evaluated with linear or nonlinear models, adjusting for other covariates, such as baseline $\log_{10}$ CFU per mL. PK/PD modeling will be performed to evaluate the relationship between drug concentrations and rate or magnitude of bacterial decline. The distributions of PK/PD parameters will be examined first and appropriate transformations will be applied if necessary. Meropenem exposure data (time>MIC, or AUC/MIC) may be added to the model, if including these data improves model fit.

- PK/PD models will be fit to assess the PK predictor that correlates best with treatment effects, as measured by EBA, and define the relationship between that parameter and effect. This PK/PD analysis will be carried out using linear or nonlinear models, adjusting for other covariates. PK/PD modeling will be performed to evaluate the relationships between drug concentrations and rate or magnitude of bacterial decline. The distributions of PK/PD parameters will be examined first and appropriate transformations will be applied if necessary.

Analyses for secondary endpoints:

- Grade 2 or higher AEs will be reported in a tabular form, by regimen.

- Descriptive statistics of $EBA_{0\text{-}14}$ TTP(rate of change in TTP in sputum over the period of Day 0 to 14) as determined by modeling analyses (as for $EBA_{0\text{-}14}$CFU) for each arm will be reported in a table. Box plots and a plot with confidence intervals will be created.

- The distribution of rifampin, meropenem, and meropenem plus amoxicillin/clavulanate MIC against *M. tuberculosis* will be reported for participants with drug-sensitive TB (Arms A and B) and drug-resistant TB (Arms C and D).

- Rifampin and meropenem PK parameters will be estimated using non-compartmental analysis and reported using descriptive statistics.

- The 2-day EBA and the EBA over other treatment time periods as measured by CFU or by TTP will be calculated in the same manner as the 14-day EBA as described above for each treatment Arm.

11.3 Sample size

For this study, the overall target sample size is 88 analyzable participants, as distributed below.

Per-arm sample sizes of 12 to 16 participants are considered standard for tuberculosis EBA studies [Boeree 2015; Heinrich 2015; Diacon 2013]. In this study, given that the MIC distribution of rifampin against *M. tuberculosis* in patients with drug-resistant TB is likely to be variable and that exposures to rifampin at a dose of 1200 mg are also highly variable, a higher number of participants, n=24, will be enrolled in Arm A; 16 participants will be enrolled in Arm B. 12 participants will be enrolled Arm C and 12 participants will be enrolled in Arm D – this will provide a sufficient range of MICs and exposures to test hypotheses related to activity of rifampin in combination with meropenem plus amoxicillin/clavulanate. 12 participants will be enrolled in Arm E and 12 participants will be enrolled in Arm F.

12 PHARMACOLOGY STUDY DESIGN

12.1 Methods and Timing for Assessing, Recording, and Analyzing PK Outcome Measures

Intensive PK sampling for rifampin and meropenem will be performed when all study drugs are at steady state (day 13, +/- one day). Blood will be collected pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post-dose (12 and 24-hour post-dose sample for rifampin measurement only (Arms A and C)). The rifampin MIC for *M. tuberculosis* for each individual will be determined and rifampin AUC/MIC for each study participant will be estimated.

12.2 PK Evaluations

PK parameters for rifampin and meropenem will be derived from plasma concentration versus time data. Samples will be drawn on a specified study day at optimized sampling times, as per section 12.1. The PK parameters to be assessed include:

- $AUC_{0\text{-}24}$ – area under the plasma concentration-time curve in one dosing interval over 24 hours;
- C_{max} – maximum observed plasma concentration;
- C_{min} – minimum observed plasma concentration;
- T_{max} – time of maximum observed plasma concentration; and
- $T_{1/2}$ – elimination half-life
- Cl – clearance for meropenem (IV formulation); CL/F, oral clearance for rifampin)

Individual participant PK parameter values will be derived by non-compartmental methods using a validated PK program. In addition, PK/PD modeling will be performed to evaluate the relationship between drug concentrations and rate or magnitude of bacterial decline. Information about the last 3 study doses prior to the PK visit will be collected. Fasted is defined as nothing by mouth except water and study medications 2 hours pre-dose and 1 hour post-dose. In addition, information that could explain unusual PK values (e.g., vomiting after the dose, not fasting when taken) or reasons for a missed sample (e.g., dropped tube, IV stopped working) will be reported.

12.3 Pharmacokinetics: Blood Collection, Processing, and Storage

Blood samples for study drug concentrations will be collected from an indwelling catheter, or, if a catheter cannot be placed successfully, by direct venipuncture. If a catheter is used for blood collection, then prior to blood sampling, the fluid in the catheter will be completely withdrawn at each sampling time and discarded. Each sample will require approximately 4 mL of blood be drawn into one Vacutainer tube. Plasma samples will be processed and frozen immediately in an upright position at -70ºC or colder, in storage boxes. These samples will be used only for PK studies. Samples will be stored for possible measurement of amoxicillin or clavulanate concentrations, should there be scientific interest and support for doing this.

12.4 Laboratory Performing the Assays

Plasma rifampin and meropenem concentrations will be determined by a validated high-performance liquid chromatography or LC-MS/MS procedure performed according to written standard operating procedures. The intraday accuracy and intraday precision will be obtained with quality control samples, which will be analyzed concurrently with each set of volunteer samples. Quality control procedures will be in place to ensure stability of sample materials.

13. HUMAN SUBJECTS PROTECTION

13.1 Institutional Review Board Involvement

This research will be conducted in compliance with the requirements of the US DHHS regulations to protect human subjects from research risk (45 CFR Part 46), in compliance with South African regulations protecting human subjects, and with oversight by the IRBs of Johns Hopkins University School of Medicine (FWA #00005752) and Pharma Ethics (FWA # 00012241). All study personnel will complete a course in protection of human subjects prior to participating in the study.

The investigator will ensure that the purpose of the study is explained to the patient and that written consent is obtained prior to participation in the study. The patient, investigator or designee, and others as required by local regulatory guidelines will sign the consent prior to entry into the study. The patient will receive a copy of his/her signed consent form. The investigator will retain a copy of the signed consent forms, which may be inspected at the monitor's or auditor's request.

The investigator will promptly report to the Ethics Committee/IRB and regulatory body of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without Ethics Committee/IRB and regulatory body approval, except where necessary to eliminate apparent immediate hazards to human subjects.

13.2 Informed Consent

Written informed consent will be obtained by trained study personnel prior to screening for inclusion and exclusion criteria. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. The informed consent process is described in detail in the "Informed Consent Process" section of "Study Procedures" (Section 9).

13.3 Confidentiality

All study records will be managed in a secure and confidential fashion. Study records will be maintained in locked cabinets, and computer records will be password protected. Access to study records will be restricted to specified study team members. The study monitor(s) or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator.

13.4 Special Populations

There will be no exclusion based on gender or ethnicity. Children will not be enrolled in this exploratory EBA study because of differences in the typical disease presentation in children versus adults; study participants must be able to produce a 16-hour sputum specimen daily for seven days and few children can do this. Pregnant women or breast-feeding women will not be enrolled since a 14-day delay in initiation of definitive TB treatment could increase the risk of TB transmission to the fetus/newborn. In addition, pregnancy may affect study drug pharmacokinetics and introduce a source of variability that cannot be adequately addressed in this small PK and safety study. It is anticipated that approximately 40% of study subjects will be women, since, historically, approximately 60% of patients with tuberculosis at clinical sites are males. The gender, ethnicity, and socioeconomic background of study subjects are expected to mirror that of the population of the hospital and clinics served, and that of the population most affected by tuberculosis. This trial will not enroll persons who are incarcerated.

13.5 Ethical Issues in Doing This Trial in South Africa

The vast majority of the global TB burden occurs in developing countries. South Africa has an exceedingly high TB case rate of approximately 600/100,000 population/year, and ranks #7 in the world based on number of incident cases. New treatment regimens that substantially improve tuberculosis treatment (shorten and/or simplify therapy) are critically needed in this setting. Therefore, it is appropriate to study novel treatments for TB and drug-resistant TB in South Africa.

13.6 Future Use of Stored Specimens

There will be potential future analysis of stored blood and urine specimens. In addition measurements of amoxicillin and/or clavulanate on plasma PK samples may be performed if there is scientific interest and resources become available.

14. MONITORING AND DATA HANDLING

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, CGP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs. Site monitoring will performed according to a written monitoring plan. Data handling and record keeping will be performed according to procedures that will be developed in a detailed Data Management Operations Manual. A participant's name will be collected only one time, and this information will be kept on a form that does not contain test results, and that is filed separately from forms that contain test results. Each participant will be assigned a unique study ID number. This number will be recorded on each data collection form and clinical specimen. Names and other obvious identifiers will not be used on data collection forms. All study records will be stored in locked files in a secure area and access will be limited to study personnel and designated regulatory personnel. All forms will be reviewed prior to data entry for accuracy, consistency, and completeness by designated study staff.

Source Documents and Access to Source Documents Appropriate records will be maintained for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The following will have access to study records: members of the study team; IRBs that review the study, the Office of Human Research Protections, the FDA, and the MCC. Authorized representatives of the sponsor and regulatory agencies will be permitted to examine study records for QA reviews, audits, and evaluation of the study safety and progress.

Data Management Responsibilities The on-site principal investigator and data manager will be responsible for accuracy, completeness, and storage of source records and data collection forms. The study team and data entry staff will review source documents to ensure accuracy and completeness. The site administrator will maintain logbooks to record dates of completed and upcoming clinic visits and specimen collections.

Data Capture Methods Hard copy CRFs will be used for data capture purposes.

Reports The timing of reports will be detailed in the Data Management Operations Manual. Briefly, safety data will be reviewed by the DSMB after 50% of the participants have been enrolled and annually thereafter; reports for the DSMB will be prepared for the DSMB according to a schedule determined at the first convened DSMB meeting. Data coding will occur at the time of data collection; ongoing logical data queries will be performed.

Study Records Retention Within 2 years of study completion, identifiers excluding study ID number will be deleted from data files. Study records will be maintained by the investigator for a minimum of 5 years following study discontinuation. The FDA will be notified prior to destruction of study records.

Protocol Deviations A protocol deviation is any noncompliance with the clinical trial protocol. As a result of deviations, corrective actions are to be developed and implemented. The site will identify and report deviations according to the guidelines of the IND sponsor and IRBs.

Quality Management Procedures for quality management will be detailed in a separate Quality Management Plan. The study site will provide direct access to all trial related facilities, source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by regulatory authorities. Following written procedures, study monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and the applicable regulatory requirements.

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APPENDIX A: SCHEDULE OF EVENTS

(SEE NEXT PAGE)

Evaluation	Screening	Enroll-ment Day -1	Day 0	Study Treatment (Days)														Dis-charge	Final Visit	Early withdrawal
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	Day 15	Day 28 +/-3	
Demographics	X																			
Documentation of HIV status	X																			
Medical/Medication History	X																			
Complete Physical Examination	X																			
Targeted Physical Examination			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Karnofsky Score	X																			
Height	X																			
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Signs/Symptoms/Diagnoses			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events assessment				X	X	X	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	X	X	X	X	X
Assignment and randomization		X	(X)																	
Complete Blood Count with Differential	X									X							X		X	X
Complete Blood Chemistry	X									X							X		X	X
CD4+ Count (HIV-infected participants)	X																			
Pregnancy Test (women)	X		X														X			
Chest X-ray	X																			
Spot Sputum for smear microscopy, Xpert MTB/RIF	X																			
Spot Sputum Collection for culture, phenotypic DST, MIC		X																		
Overnight Sputum Collection		X	X	X	X	X	X		X		X		X		X		X			
EBA Analysis by Solid Culture CFU and Liquid MGIT Culture		X	X	X	X	X	X		X		X		X		X		X			
Blood and Urine Retention Sampling			X			X				X							X			X
Pharmacokinetic Sampling																X				
Hospitalization	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Counseling about study procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			

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