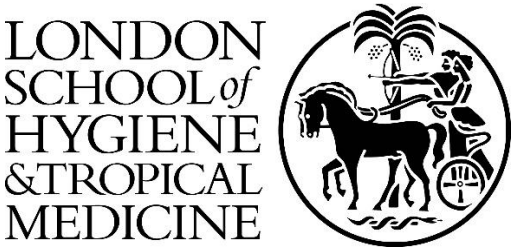

Short, effective and safe all-oral treatment for rifampicin resistant tuberculosis

The TB-PRACTECAL trial and its drugs pharmacokinetics

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Declaration

I, Bern-Thomas Bernard Nyang'wa, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Background

Rifampicin resistant tuberculosis (RR-TB) causes disease in 410,000 people annually. Treatment of RR-TB used to be lengthy, complex, ineffective, poorly tolerated and expensive. We aimed to identify short, effective and safe all oral regimen(s) for the treatment of pulmonary RR-TB. In addition, we aimed to investigate the relationship between the patients' exposure to anti-TB drugs and treatment outcomes.

Methods

An open label, randomised, controlled, multi-arm, multicentre, non-inferiority trial was conducted in Uzbekistan, Belarus, and South Africa. Participants were randomised in a 1:1:1:1 ratio to receive standard of care (SoC); 24-week oral bedaquiline, pretomanid, and linezolid (BPaL); BPaL plus clofazimine (BPaLC); or BPaL plus moxifloxacin (BPaLM) in stage one of the trial and in a 1:1 ratio to receive SoC or BPaLM in stage two of the trial. The primary outcome was the percentage of participants with a composite unfavourable outcome (death, treatment failure, treatment discontinuation, recurrence or loss to follow-up) at 72 weeks post randomisation. A non-inferiority margin of 12% and a power of 85% were assumed.

In the pharmacokinetic study, blood samples were collected on Day 1 (0, 2 and 23 hours), Weeks 8 (predose, 6.5 and 23 hours), 12, 16, 20, 24, 32 and 72 post randomisation visits from a subset of participants randomised to the interventional arms only. Drug concentrations were quantified in a Good Clinical Practice (GCP) laboratory using a high-performance liquid chromatography-tandem mass spectrometry. nlmixr2, an open-source R package was used for population pharmacokinetic (PK) modelling. Probability of target attainment for concentration dependent and time dependent indices were area under the concentration-time curve from zero to twenty four hours over minimum inhibitory concentration (AUC_{0-24} /MIC) and percentage of the dosing interval during which the plasma concentration exceeds the MIC (%T>MIC).

Results

552 participants were enrolled in the randomised controlled trial (RCT), 41% were female, with a median age of 35 years. 28% were living with HIV, 65% had smear positive, 61% had cavities on chest x-ray and 89% were culture positive. In stage 1, BPaLM was chosen due to higher culture-conversion rates at 8 weeks (BPaLM 77%, BPaLC 67%, and BPaL 46%). The trial was discontinued early for benefit. The primary unfavourable outcomes proportions at 72 weeks post randomisation were 41%, 12%, 23% and 14% for SoC, BPaLM, BPaLC and BPaL arms respectively. 23%, 30% and 24% of participants receiving BPaLM, BPaLC and BPaL respectively, had adverse events of grade 3 or higher or serious adverse events, compared with 48% of participants receiving standard care.

A one-compartment, first order absorption and elimination disposition model with fat-free mass allometric scaling and Caucasian race covariate on clearance best described the linezolid pharmacokinetics. The 600mg dose probability of free drug area under the concentration-time curve from zero to twenty four hours of free drug ($fAUC_{0-24}$) divided by the minimum inhibitory concentration (MIC) target of 119 was reached for MIC of 0.25 mg/L.

A one-compartment first order absorption and elimination model with allometric scaling of fat-free mass on both clearance and volume of distribution best characterised pretomanid pharmacokinetics. Virtually all patients on a 200mg daily had drug exposures above 77% of the dosing interval during which the unbound drug plasma concentration exceeds the MIC ($fT>MIC$) target and at least 96% would have been above the 167 $fAUC_{0-24}$/MIC target.

A two-compartment first order absorption and elimination body weight allometric scaling model with a lag time absorption parameter best described the pharmacokinetics of clofazimine. Using 100mg daily, the probability %T>MIC target could be achieved at MIC of 0.5mg/L.

Bedaquiline population pharmacokinetics was best described by a three-compartment model with fixed transit compartments with BMI allometry. When dosed at 400mg daily for two weeks followed by 200mg three times a week, probability of target attainment above 90% was only achieved for MICs below 0.063mg/L.

Conclusion

BPaLM was both safer and more efficacious than the then SoC. An optimal design-led sparse sampling schedule allowed for satisfactory population pharmacokinetic modelling for linezolid, pretomanid, clofazimine and bedaquiline. Further pharmacodynamic analyses are recommended to elucidate the contribution of each drug to the trial outcomes.

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Preface

I present this thesis as a research paper style thesis.

Two of the eight chapters comprise a total of four research papers that have been published in peer-reviewed journals and are indicated in the table of contents. Cover sheets are provided with each paper that detail publication details and author contributions.

In addition to the introduction and discussion chapters, four chapters are each a draft paper for publication.

Acknowledgements

I feel blessed and favoured to have had the opportunity to make a small contribution to alleviating suffering from tuberculosis through the TB-PRACTECAL trial.

To Dr Phillip du Cros, Dr Leslie Shanks, Dr Corinne Merle, Prof Katherine Fielding, Prof Dave Moore who trusted me to lead the project and supported me all the way, I am very grateful.

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To Dr Maggie Nyang'wa, my wifey, you finally have a learned husband. Thank you for pushing me to enrol and finish this PhD, for absorbing all the PRACTECAL shocks and standing by me, I'll forever be grateful and appreciative of your love. To Maya Nyang'wa, my daughter, for you the sky is a target to surpass and not the limit. To my son, Miko Nyang'wa, this is what a 'bern' does, permit that lamp to shine bright.

I dedicate this PhD thesis to Mr Bernard Robin Nyang'wa, you didn't get to experience it enough but I'm sure you saw it way before I did. Rest in peace dad.

Table of abbreviations

ADDL	additional identical dose given derived
AE	adverse event
AESI	adverse event of special interest
AMT	dosing record
AUC	area under the curve
B	bedaquiline
BMI	body mass index
BPaL	regimen consisting of bedaquiline, pretomanid and linezolid
BPaLC	regimen consisting of bedaquiline, pretomanid, linezolid and clofazimine
BPaLM	regimen consisting of bedaquiline, pretomanid, linezolid and moxifloxacin
Cfz	clofazimine
CL/F	apparent clearance
C_{max}	maximum concentration
C_{min}	minimum concentration
CMT	compartment code for observation or dosing record
CRF	clinical research form
DS-TB	drug susceptible TB
DV	Measured drug concentration or dependent variable
EMA	European Medicines Agency
F	bioavailability
FDA	(US) Food and Drug Authority
FFM	fat-free mass
FIM	Fisher information matrix
GCLP	good clinical laboratory practice
GOF	goodness of fit
HPLC	higher performance liquid chromatography
ICF	informed consent form

ID	Subject identification
II	interdose interval
Ka	absorption rate constant
LLQ	lower limit of quantification
LPA	line probe assay
Lzd	linezolid
MDR-TB	multidrug resistant TB
MDV	Missing data value
Mfx	moxifloxacin
MIC	minimum inhibitory concentration
mITT	modified intention to treat population
MS	mass spectrometry
Mtb	Mycobacterium tuberculosis
NAATs	nucleic acid amplification tests
OFV	objective function value
Pa	pretomanid
PD	pharmacodynamics
PDATE	Date and actual time of sample collection
PK	pharmacokinetics
popPK	population pharmacokinetics
PTA	probability of target achievement
PTIME	Identification of the timepoint of sample collection
Q	intercompartment clearance
RR-TB	rifampicin resistant TB
RSE	relative standard error
SoC	Standard of care
SSE	stochastic simulation and estimation
START	The date and time of first dose

$T_{1/2}$	half-life
TB	Tuberculosis
T_{max}	time at maximum concentration
Vc	volume of central compartment
VISIT	Identification of the timepoint of visit number
Vp	volume of peripheral compartment
WHO	World Health Organization
XDR-TB	extensively drug-resistant TB

"What counts in life is not the mere fact that we have lived. It is what difference we have made to the lives of others that will determine the significance of the life we lead."

Nelson Rolihlahla Mandela

1918 – 2013

Chapter 1: General introduction

This chapter consists of a brief introduction to tuberculosis, including its pathogenesis, clinical features, laboratory diagnostic options, treatment options and changes over time, resistance development and amplification and global epidemiology. Aims and objectives of thesis are presented and the PhD body of work, thesis structure, publications and related outputs are described.

1.1. Tuberculosis

Tuberculosis (TB) is a disease caused by the bacillus Mycobacterium tuberculosis (Mtb) which primarily infects the lungs. There is evidence of TB dating back more than 4,000 years ago in Egyptian mummies and depicted in Egyptian art (1).

1.1.1. Pathogenesis

Transmission of Mtb is airborne, first infecting alveolar macrophages and later in an innate immune response, interstitial macrophages, dendritic cells and neutrophils. An adaptive immune response involving T-cells, B-cells and macrophages form a granuloma where bacterial replication is contained, and disease progression halted (2). For some partially understood reasons, whether due to comorbidity with Human immune deficiency syndrome, malnutrition and Diabetes Mellitus, infancy or overwhelming bacillary population as in prisons, patients develop active TB. Necrotic TB granuloma

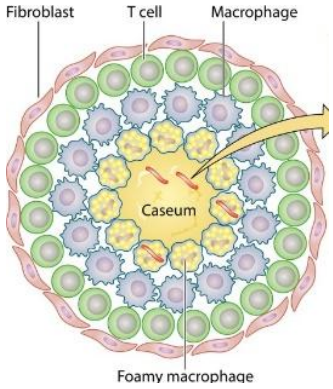

Figure 1.1: components of a necrotic granuloma. adapted from Jansy Sarathy and Veronique Dartois (2020)

with a caseating appearance (fig 1.1) (3) are the commonest form of active TB and plays a significant role in transmission..

Evolution in the understanding of TB pathogenesis has accelerated in the 21st century, moving from a binary paradigm of latent TB and active TB disease (4, 5) to one that identifies additional discrete intermediate steps of incipient and subclinical disease shown in figure 1.2 (6, 7). Incipient TB infection is an infection with viable M. tuberculosis bacteria that is likely to progress to active disease in the absence of further intervention but has not yet induced clinical symptoms, radiographic abnormalities, or microbiologic evidence consistent with active TB disease. Subclinical TB disease is disease due to viable M.tb bacteria that does not cause clinical TB-related symptoms but causes other abnormalities that can be detected using existing radiologic or microbiologic assays (8).

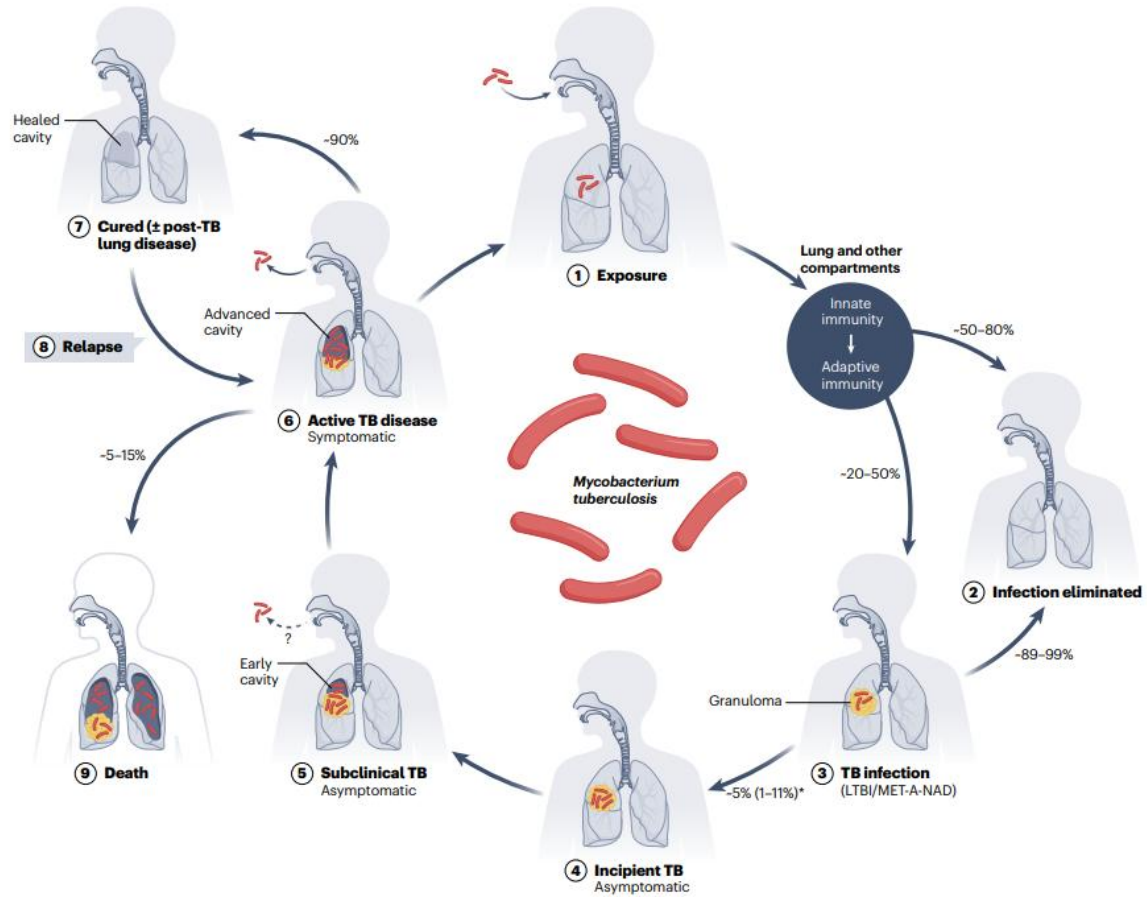

Figure 1.2: The life cycle of Mycobacterium tuberculosis. Adapted from Dheda et al. Nature reviews Disease Primers (2024).

1.1.2. Clinical features

In most people, exposure to Mtb does not result in any clinical symptoms (9). To those who develop active disease (TB), it commonly presents with cough, fever, night sweats and loss of weight (10). Other organ-specific signs and symptoms may be observed such as loss of consciousness due to meningoencephalitis or tuberculoma, gibbus due to spinal osteomyelitis, cardiomegaly due to pericardial effusion or neck swelling due to cervical lymphadenopathy (11).

1.1.3. Diagnosis

Active TB can be detected through light microscopy, nucleic acid amplification tests (NAATs), cultures and genome sequencing (12, 13). Use of Ziehl-Neelsen (ZN) or auramine smear staining for observation under light microscopy is not preferred primarily due to the low sensitivity. WHO recommends that people presenting with signs and symptoms of TB should first have a rapid molecular test with drug resistance detection (14). These are NAAT tests such as Xpert MTB/RIF and Truenat MTB-RIF which identity Mtb and resistance to rifampicin. Loop-mediated isothermal amplification (LAMP) and lateral flow urine lipoarabinomannan (TB-LAM) can also be used in specific circumstances but do not identify resistance. Follow-on tests for diagnosing resistance to quinolones and aminoglycosides include line probe assays (LPA) and Xpert MTB/XDR. Cultivation of Mtb remains the gold standard method of diagnosis, it is however laborious and slow requiring up to 8 weeks before confirming a negative result. Genome sequencing provides molecular profiles of drug resistance within a single analysis, although this is currently not widely available (15).

1.1.4. Resistance development

The traditional understanding of resistance development and amplification is that, especially in a caesium where millions of bacilli reside, spontaneous genetic mutations result in subpopulations that are resistant to some drugs (16). Under drug pressure, these resistant bacilli become the predominant population causing disease and transmitted to other people. Other mechanisms include mutation to genes that are not in the drug's mechanism of action pathway e.g. upregulation of efflux pumps, epigenetic

mechanisms, site of infection pharmacokinetic variability and psychosocial and programmatic factors (see figure 1.3) (7).

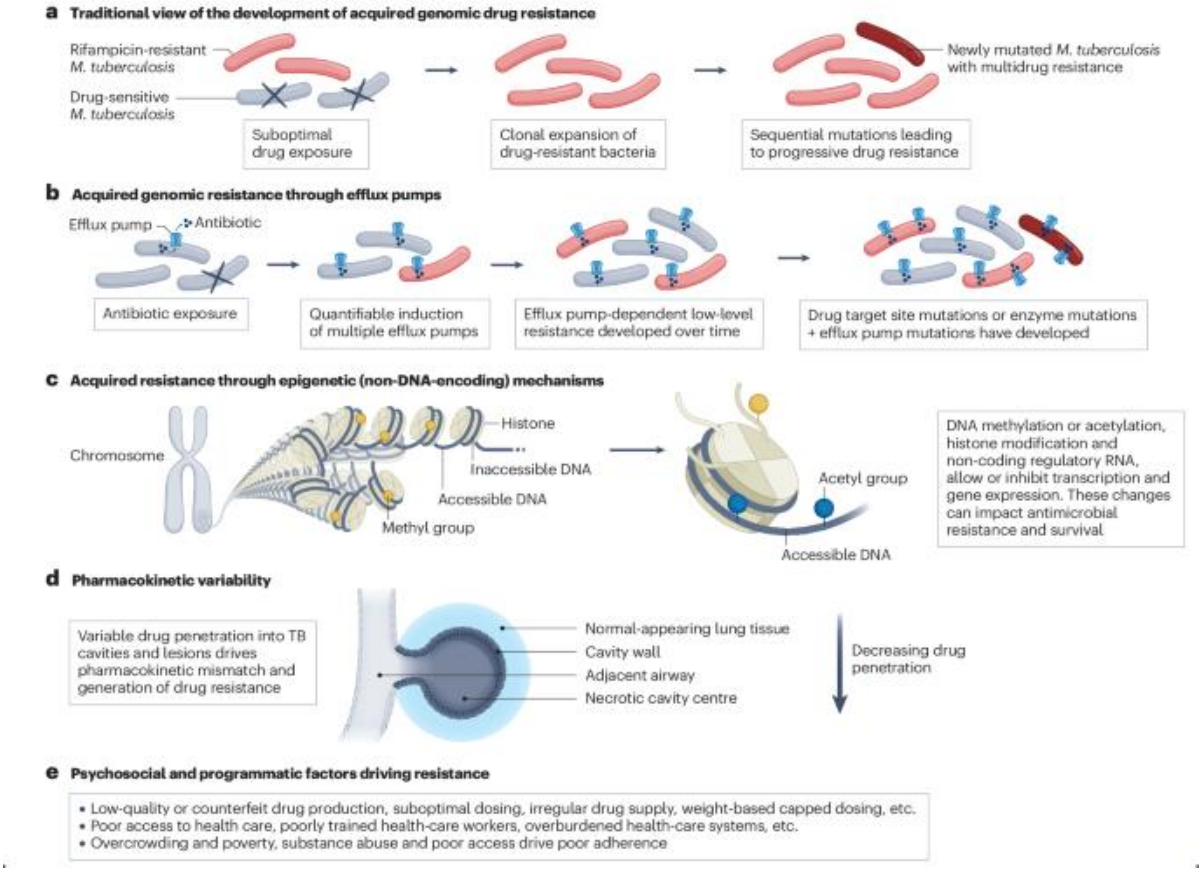

Figure 1.3: Mechanisms of resistance development. Adapted from Dheda et al. Nature reviews Disease Primers (2024).

1.1.5. Global burden

Tuberculosis remains one of the deadliest infectious diseases globally. The World Health Organization estimates that in 2022 alone, 1.3 million people died of TB. In this year, there were 133 incident cases per 100,000 population, totalling to an estimated 10.6 million people falling ill to TB disease. 410,000 of the persons with TB, developed multidrug-resistant / rifampicin resistant (MDR/RR)-TB, this was 3.3% of people with no previous history of TB and 17% of people previously treated for TB (17). Despite RR-TB being a global epidemic, eight countries report over 50% of estimated incident cases (see Fig 1.4).

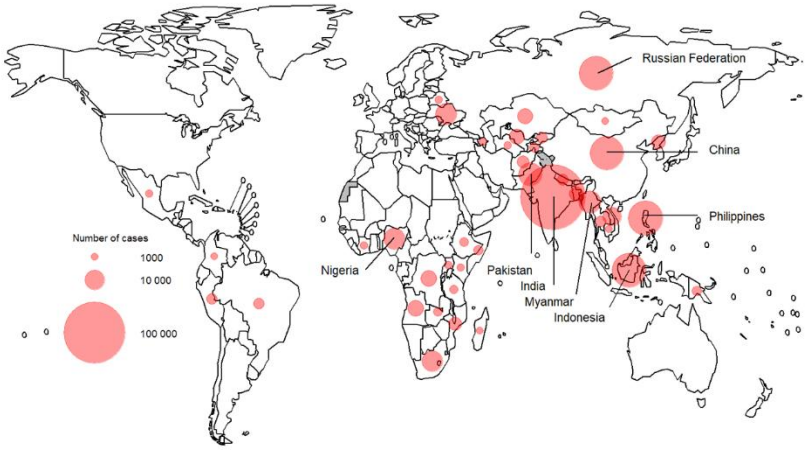

Figure 1.4: The eight countries with at least 1,000 incident cases ranked in descending order of their total number of MDR/RR-TB incident cases in 2022: India, the Philippines, the Russian Federation, Indonesia, China, Pakistan, Myanmar and Nigeria (17). Adapted from WHO Global TB report 2023

Although RR-TB constitutes a small proportion of incident TB, it has relatively higher burden on individuals and health systems. A person developing RR-TB has a 34% higher loss in disability adjusted life years at 17.3 in comparison to 12.9 in drug susceptible (DS) TB (18). 83% of patients with drug resistant(DR) -TB and their households faced catastrophic costs (>20% of annual household income), compared with 49% of those with DS-TB (17).

1.1.6. Treatment

Drugs for treating TB are often categorised into first line drugs for the treatment of drug susceptible-TB and second line drugs for the treatment of drug-resistant TB. Drug susceptible TB is usually treated with rifampicin(R), isoniazid(H), pyrazinamide(Z) and ethambutol(E) for two months followed by a continuation phase of rifampicin and isoniazid for another four months (2HRZE/4RH). Children and adolescents with non-severe TB may shorten the continuation phase to two months (2HRZE/2RH) (19, 20). These treatments are relatively well tolerated when compared to RR-TB treatment and success in programmatic settings is around 85% (17). Another 4-month regimen of isoniazid, rifapentine(P), moxifloxacin(M) and pyrazinamide (2HPMZ/2HPM) may be used in adolescents and adults (21).

Drug resistant TB is categorised into rifampicin resistant (RR-TB), multidrug resistant (MDR-TB), pre-extensively drug resistant (Pre-XDR TB) and extensively drug resistant (XDR-TB), see figure 1.5 for the resistant drugs in each category. These categorisations are used for treatment choices. RR-TB is considered interchangeable with MDR-TB due to the wide use of GeneXpert diagnosis which cannot differentiate whether the patient has isoniazid resistance as well, and the treatment is often the same (22).

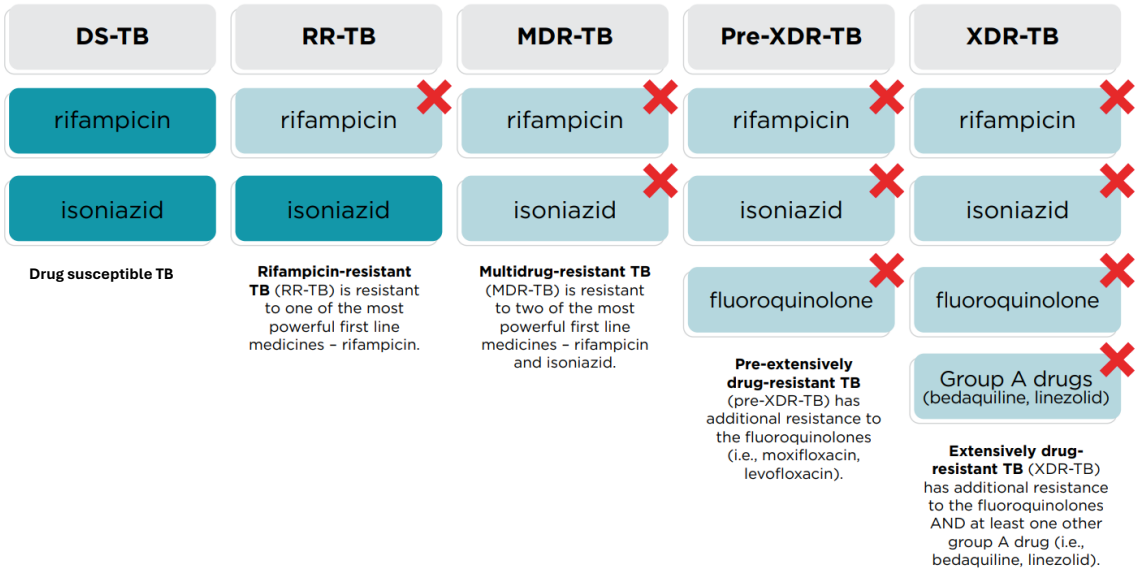

Figure 1.5: TB resistance categorisation. Adapted from 'An activist's guide to shorter treatment for drug-resistant tuberculosis'. Treatment Action Group. 2023(23).

Treatment of multidrug resistant TB as a public health policy called DOTS-plus, was adopted just before the turn of the millennium (23) but not without opposition (24). The regimens varied but commonly consisted of an aminoglycoside (streptomycin, kanamycin, capreomycin, amikacin), a quinolone (ofloxacin or ciprofloxacin), cycloserine, ethionamide, para-aminosalicylic acid (PAS), pyrazinamide and ethambutol for 24-36 months (25). The only improvements up to 2010 was that fourth-generation quinolones (levofloxacin, moxifloxacin (Mfx/M) and gatifloxacin) were preferred to ciprofloxacin and ofloxacin (26). The first major change was the development of shorter regimens, lasting 9-11 months, which were first reported in 2012 (27, 28). At the same time bedaquiline (B) was approved for the treatment of MDR-TB, however its use at programmatic level was delayed and only recommended in 2016 (29). Linezolid (Lzd/L) was promoted and injectable aminoglycosides except amikacin received negative

recommendations (30, 31). Current RR-TB treatment recommendations include the 6-month bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) regimen, the 9-month all-oral regimen and the 18-20 month longer regimen. The biggest single step change for RR/MDR TB treatment has been the preference for use of the six-months all-oral regimens consisting of bedaquiline, pretomanid, linezolid and moxifloxacin (32).

As the TB-PRACTECAL trial was started in 2017, treatment of RR/MDR-TB remained lengthy, complex, ineffective, poorly tolerated and expensive (33). Globally, successful outcomes in patients started on treatment increased from 50% in 2012 to 63% in 2020 (17). Poor effectiveness combined with high costs and difficulty with implementation prevented many national TB programs from offering treatment for MDR-TB (34). This in turn fuelled the spread of MDR-TB infections (35)

1.1.7. Rationale for the PRACTECAL trial choices:

Since sustained cure of tuberculosis has only been achieved by combinations of drugs, the trial primarily evaluated regimens rather than individual drugs. The choice of the regimens to be studied and design of the trial was strongly influenced by the following eight key principles which have previously been published for designing future MDR-TB treatment regimens: It should contain at least one new class of drug; It should be broadly applicable for use against MDR and XDR Mycobacterium tuberculosis complex strains; It should contain three to five effective drugs, each from a different drug class; It should have an exclusively oral delivery; It should have a simple dosing schedule; It should have a good side-effect profile that allows limited monitoring; It should have a maximum duration of six months; It should have minimal interaction with antiretroviral drugs (33).

Rationale for composition of regimens

The investigational arms were based on a backbone of B, Pretomanid (Pa) and Lzd. The combination of B and Pa was selected on the basis of murine model studies and the clinical combinations in the TB Alliance studies NC-001 (36) and NC-003 (37). Lzd was included in all the regimens on the basis of the murine studies combining B, Pa and Lzd, and the improved outcomes in XDR-TB patients when Lzd was added to existing therapy (38). Regimens including these three drugs, which are from different drug classes with

different mechanisms of action, and likely low rates of baseline population-level resistance in TB were theorised to be effective against both MDR and XDR TB strains.

In a murine aerosol infection model of TB, the combination of bedaquiline, pretomanid and moxifloxacin was more effective at reducing relapse after 4 months treatment than standard treatment with rifampicin, isoniazid and pyrazinamide. Studies in the murine model of Lzd in combination with bedaquiline and pretomanid showed marked reductions in colony forming units after 1-3 months of treatment and cured mice 1- to 2-months faster than the standard treatment of isoniazid, rifampicin and pyrazinamide (39).

In the NC-001 trial, a 14 day early bactericidal activity (EBA) study, 85 patients were randomised to standard treatment (RHZE) or 5 treatment arms including a bedaquiline-pretomanid arm. The bedaquiline-pretomanid arm showed significantly greater reduction in cultured colony counts than bedaquiline alone at 2 days, but slightly lower activity than standard treatment at day 14. There were no serious adverse events among patients on the bedaquiline-pretomanid arm, with 1 patient withdrawn due to a grade 3 elevation of ALT.

In the NC-003 trial, a 14-day EBA study of 105 patients randomised to 2 monotherapy and 4 combinations including bedaquiline, pretomanid, pyrazinamide and clofazimine and standard treatment (RHZE). This study included 3 arms with the combination bedaquiline-pretomanid: B-Pa-Z, B-Pa-Z-Cfz, and B-Pa-Cfz. All 3 regimens showed significant bactericidal activity. The addition of clofazimine did not increase EBA activity. The bedaquiline-pretomanid-pyrazinamide arm demonstrated a rate of decrease in log colony-forming count (CFU) as good as the standard treatment. There were no major serious adverse events in these 3 interventional arms. One subject was withdrawn due to raised liver enzymes in the B-PA-Z arm. There was no prolongation of corrected QT beyond 500 ms in the bedaquiline-pretomanid arms, although in the B-Pa-Cfz arm 2 subjects had Bazett's corrected QT increase by more than 60 ms.

Rationale for the chosen duration of investigational Arms

Predicting the optimal duration for a new clinical regimen is challenging. Murine models can be helpful in studying specific factors of drugs and regimens such as bactericidal

activity and sterilising ability, however there are host differences between TB in mice and in humans that may mean that predictions of duration may not be directly comparable (40). The six months duration of treatment for the investigational arms was chosen based on:

1) Preclinical studies evidence:

The combination of B, Pa and Lzd has greater sterilizing activity than standard DS-TB treatment (RHZ) and achieves relapse-free cure of mice 1-2 months faster when using doses in mice reasonably equivalent to those administered in humans. 6 months treatment is therefore a conservative choice but also takes into account that due to toxicity; some of the doses (especially Lzd) may not be achieved through out the duration of the treatment.

2) Comparison to 'current' DS-TB regimen in humans

The investigational arms included at least three agents that TB strains were likely to be sensitive to for the entire duration of treatment. This made the patients comparable to those being treated for DS-TB. And the current evidence at the time, demonstrated that DS-TB patients could be successfully treated with a 6 months treatment regimen. This comparison could be questioned due to the differences in the properties of the drugs included in the regimens. However, the early bactericidal activity of Pa, Lzd and Mfx, the sterilising ability of B, Cfz and Lzd including in chronic states, may be comparable to those of R, H, Z, E. Perhaps more important, were the synergistic activity of B, Pa and Lzd as a back bone and in addition to the synergistic value of Mfx when added to B and Pa.

3) Approved duration of use for study drugs

Bedaquiline is registered for 6 months treatment and the phase 3 programme for Pa was studying it in regimens of 4 and 6 months. This choice of duration ensured that the successful regimens will not necessarily need further length of administration label amendment.

Furthermore, longer duration may be considered necessary to achieve relapse free cure when focusing on dormant or non-replicating mycobacteria (41). Both bedaquiline and pretomanid have shown good activity in models of non-replicating mycobacteria

suggesting that a regimen containing the combination of these drugs could have the properties necessary for a short 6 month treatment for MDR TB (REF: Grant SS, Kawate). The relapse rate and the duration of TB treatment required to prevent relapse are associated with the mycobacterial load at baseline, and with the presence of cavities.

1.2. TB drugs pharmacokinetics

A summary of the pharmacokinetics – absorption, distribution, metabolism and elimination, of each drug studied in the PhD formed the basis of the development of PRACTECAL PKPD study. The updated summaries are presented below.

1.2.1. Bedaquiline

Bedaquiline is a diarylquinoline antimycobacterial which inhibits the proton pump of mycobaterial ATP synthase. It is given orally at a dose of 400mg daily for 2 weeks and then 200mg three times a week for 22 weeks. B is well-absorbed with a T_{max} of 5 h. The C_{max} is 3.060 mg/L at week 2 and 1.838 mg/L at week 24. Administration of a high fat meal increases bioavailability by 95%. It is more than 99.9% protein bound at a concentration of >5mg/L. It is metabolized by oxidative metabolism via the CYP3A4 isoenzyme. The average terminal elimination half-life is 132 days. Faecal route is the major route of elimination, negligible amount of unchanged drug is found in urine (42-44).

1.2.2. Pretomanid

Pretomanid is a nitroimidazooxazine antimycobacterial approved for the treatment of TB. It inhibits mycolic acid biosynthesis, thus disrupting cell wall production in actively replicating Mtb. It also kills non-replicating bacteria in anaerobic environments by generating reactive nitrogen species including nitric oxide(45). At an oral dose of 200 mg, steady state PK parameters are as follows: C_{max} 1.7 mg/L, T_{max} of 4.5 hours, T1/2 16 hours. A high-fat, high-calorie meal increased C_{max} by 76% and AUC by 88% when compared to the fasting state. Pretomanid is extensively metabolized through reductive and oxidative metabolism but no identified major pathway. Only 20% is metabolised through cytochrome P450-3A. 1% appears in urine as unchanged pretomanid (46-49).

1.2.3. Linezolid

Linezolid (Lzd) is an oxazolidinone class antimicrobial approved for Gram-positive bacterial infections. It's mechanism of action is through inhibiting ribosomal 8 protein synthesis by binding to the 23S RNA peptidyl transferase centre of the 50S subunit of the prokaryotic ribosome(50). It is highly bioavailable and rapidly absorbed as an oral tablet of 600mg reaching peak plasma concentrations of 12.7 mg/L at a T_{max} of 1.3 hours. It is hepatically metabolized, and its clearance varies with age and gender. It has a half-life of 4 hours. Nonrenal clearance accounts for 65% of linezolid clearance. 30% of the dose appears in the urine as linezolid. The mean renal clearance of linezolid is 40 mL/min (51-53).

1.2.4. Clofazimine

Clofazimine (Cfz) is a lipophilic riminophenazine licensed for treatment of leprosy. Several mechanisms of action have been postulated which may predominate depending on the specific physiological environment, some of these include intracellular redox cycling, interfering with potassium uptake in membrane phospholipids and anti-inflammatory activity through inhibition of T-lymphocytes activation and proliferation. Oral administration of clofazimine 100mg daily in leprosy patients results in average plasma levels of 0.7 mg/L. High fat food increases bioavailability by 45%. When dosed at 300mg for the first three days and then 100mg for the remaining 11 days, the C_{min} and C_{max} at day 14 are 0.153mg/L and 0.232mg/L respectively. Its $T_{1/2}$ is 25 days. Clofazimine is partially metabolised in the liver, but the full scope of its metabolic pathways is not known. Negligible amount of parent drug or metabolites are found in urine; however, a significant amount is found in faeces (37, 54-57).

1.3. Aims and objectives

The overall aim of this thesis was to identify short, effective and safe all oral regimen(s) for the treatment of pulmonary rifampicin resistant tuberculosis.

The thesis objectives were:

Develop and implement a pragmatic clinical trial for a short, effective and less toxic regimen(s) for rifampicin resistant tuberculosis (TB-PRACTECAL).

TB-PRACTECAL trial was a multicentre, multistage, open label, phase 2-3 randomised controlled trial aimed at evaluating 24 week, exclusively oral regimens for the treatment of microbiologically confirmed pulmonary RR-TB. The study's primary objectives were:

Stage 1: Identify regimens containing bedaquiline and pretomanid for further evaluation based on safety and efficacy outcomes after 8 weeks of treatment.

Stage 2: Evaluate the safety and efficacy of the investigational regimens containing bedaquiline and pretomanid compared with the Standard of care at 72 weeks post randomisation.

The trial aimed to recruit 630 adolescents and adults from Uzbekistan, Belarus and South Africa. It was registered with the ClinicalTrials.gov with identifier number NCT02589782. Details of the TB-PRACTECAL trial rationale, design and results are reported in chapter 2.

Develop and implement a population pharmacokinetic and pharmacodynamic study of the investigational drugs used in the TB-PRACTECAL trial (PRACTECAL-PKPD)

The PRATECAL-PKPD sub-study was conceived so that If the TB-PRACTECAL RCT identified successful regimens, the study would provide explanatory evidence to why the tested regimens at the chosen doses and administration scenario were efficacious and add to its evidence for global policy change. The study would have, in the situation where the regimens had not been shown to be non-inferior, allow the understanding of whether variability of particular drug exposures could have played a part in the efficacy or safety outcomes and make appropriate recommendations.

The study aimed to investigate the relationship between the patients' exposure to anti-TB drugs in the TB-PRACTECAL trial investigational regimens and their respective treatment outcomes. The study's primary objective was to measure the plasma concentrations of pretomanid, linezolid, bedaquiline, clofazimine and moxifloxacin in a subset of patients in the TB-PRACTECAL trial and using population PK models, estimate the population exposure metrics (C_{min}, C_{mean}, C_{max}, area under the curve (AUC)) for the individual drugs in the TB-PRACTECAL trial.

We aimed to recruit up to 240 participants. The study was registered with the ClinicalTrials.gov with identifier number NCT04081077. Details of PRACTECAL-PKPD trial rationale and design are reported in chapter 3.

1.4. Overview of the PhD body of work

The PhD has been conducted as part of the PRACTECAL research project summarised in figure 1.6. From registration into the PhD in February 2017 to date, I have been the Chief Investigator of the TB-PRACTECAL trial, and it is within this role that I have conducted the various studies for this PhD.

Part A: The TB-PRACTECAL randomised controlled trial

I led the conceptualisation of the TB-PRACTECAL randomised controlled trial, development of the trial protocol including chairing the protocol writing committee, oversaw the implementation of the protocol, data collection, data analysis, interpretation and results communication.

I steered the research project, with input from key stake holders (research management group, site investigators, trial steering committee, data and safety monitoring board and scientific advisory committee) and was the principal decision maker on the study implementation choices (site selection, clinical management guidance, data collection tools, quality assurance approaches etc), analysis plan and manuscript content.

This body of work is reported in the format of a methods paper, an interim and a final results paper merged into Chapter 2.

Part B: The PRACTECAL pharmacokinetic and pharmacodynamic (PKPD) study

The PRACTECAL-PKPD was one of the sub-studies of the TB-PRACTECAL trial. I conceptualised and hence developed the PRACTECAL-PKPD study protocol and data collection tools (Kobo database and clinical research forms - CRFs), project administration and supervision through identification of study sites, obtaining ethics approvals, providing training and oversight for the implementation of the study. I conducted the data analysis, population pharmacokinetic modelling, target attainment analyses and reporting.

Study data was collected by site investigators in Belarus and South Africa, bioanalysis was conducted by the University of Liverpool Bioanalytical facility team, Ilaria Motta supported data curation, Zhonghui Huang wrote the pop PK code and ran the models for

the pretomanid, clofazimine and bedaquiline. I defined the pop PK methodology and sources of data, wrote and run the code for linezolid, reviewed the drafted model code for bedaquiline, pretomanid and clofazimine and took the decisions for each step of model development for all drugs.

This body of work is reported in chapters three to seven. Chapter 3 is the methods chapter, consisting of a PRACTECAL-PKPD study protocol publication and detailed methodology for the population pharmacokinetics and PKPD target attainment analyses.

The linezolid, pretomanid, clofazimine and bedaquiline population pharmacokinetics and probability target attainment are reported in Chapter 4, Chapter 5, Chapter 6, and Chapter 7 respectively.

1.5. Related research (outside the scope of this PhD)

Moxifloxacin population pharmacokinetics, multi-drug modelling exploring the pharmacokinetics-pharmacodynamic and pharmacotoxicity relationships of bedaquiline, pretomanid, linezolid, moxifloxacin and clofazimine to mycobacteriology and safety data in the TB-PRACTECAL trial will be developed beyond the scope of the PhD.

The following three sub-studies of the TB-PRACTECAL trial complemented my development in TB drugs pharmacology but are not reported in detail in the thesis:

The PRACTECAL-VAMS - the volumetric absorptive microsampling study which is aimed at determining the accuracy of anti-TB drugs quantification using dried blood collection method (VAMS) compared to traditional liquid whole blood for five investigational MDR-TB drugs used within the framework of the TB-PRACTECAL Clinical Trial.

The PRACTECAL-HAIR – aims to assess the potential of hair drug levels to objectively monitor long-term adherence and predict treatment outcomes in patients participating in the TB-PRACTECAL trial.

The PRACTECAL-PGx – The purpose of the study is to evaluate if genetic make-up could predict exposure to anti-TB drugs and MDR/RR-TB treatment outcome among MDR/RR-TB patients. More specifically, the study aims to explore the role

of specific human genes and SNPs on bedaquiline, pretomanid, linezolid, clofazimine and moxifloxacin drugs concentrations and treatment outcomes among MDR/RR-TB patients. The preliminary analyses were conducted as part of an MSc project which I co-supervised.

In support to the overall TB-PRACTECAL ambitions, I developed the concept of and identified the principal investigators for the economic evaluation (PRACTECAL-EE) and patient reported outcomes (PRACTECAL-PRO). I continued to provide oversight of these two studies by supporting the teams implementing them, contributed to the interpretation of the data analysis and the communication of the results. Both studies' results manuscripts are undergoing peer review for publication.

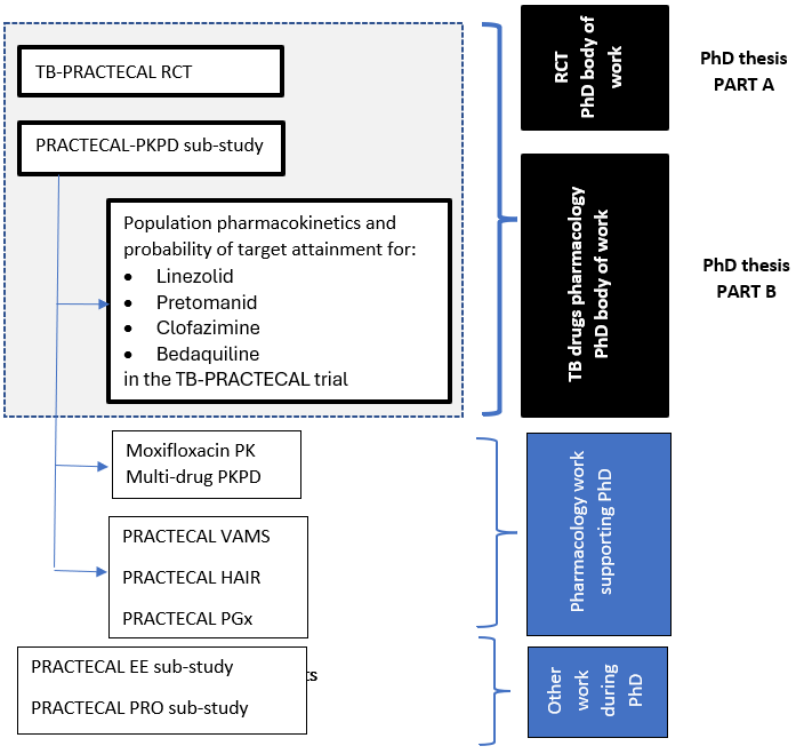

Figure 1.6: PRACTECAL research portfolio overview

1.6. PhD publications and related outputs

List of peer-reviewed publications included in this thesis.

[Chapter 2: TB-PRACTECAL trial]

1. Berry C, du Cros P, Fielding K, Gajewski S, Kazounis E, McHugh TD, Merle C, Motta I, Moore DAJ, Nyang'wa BT. TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II-III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis. Trials. 2022 Jun 13;23(1):484. doi: 10.1186/s13063-022-06331-8.

2. Nyang'wa BT, Berry C, Kazounis E, Motta I, Parpieva N, Tigay Z, Solodovnikova V, Liverko I, Moodliar R, Dodd M, Ngubane N, Rassool M, McHugh TD, Spigelman M, Moore DAJ, Ritmeijer K, du Cros P, Fielding K; TB-PRACTECAL Study Collaborators. A 24-Week, All-Oral Regimen for Rifampin-Resistant Tuberculosis. N Engl J Med. 2022 Dec 22;387(25):2331-2343. doi:10.1056/NEJMoa2117166.

3. Nyang'wa BT, Berry C, Kazounis E, Motta I, Parpieva N, Tigay Z, Moodliar R, Dodd M, Solodovnikova V, Liverko I, Rajaram S, Rassool M, McHugh T, Spigelman M, Moore DA, Ritmeijer K, du Cros P, Fielding K; TB-PRACTECAL team. Short oral regimens for pulmonary rifampicin-resistant tuberculosis (TB-PRACTECAL): an open-label, randomised, controlled, phase 2B-3, multi-arm, multicentre, non-inferiority trial. Lancet Respir Med. 2023 Nov 15:S2213-2600(23)00389-2. doi: 10.1016/S2213-2600(23)00389-2.

[Chapter 3: Methods]

4. Nyang'wa BT, Kloprogge F, Moore DAJ, Bustinduy A, Motta I, Berry C, Davies GR. Population pharmacokinetics and pharmacodynamics of investigational regimens' drugs in the TB-PRACTECAL clinical trial (the PRACTECAL-PKPD study): a prospective nested study protocol in a randomised controlled trial. BMJ Open. 2021 Sep 6;11(9):e047185. doi: 10.1136/bmjopen-2020-047185.

Related peer-reviewed outputs that I co-authored included in this thesis

[Conference poster in Appendix]

5. Using an Optimal Design approach to efficiently design a PKPD study of multiple anti-TB drugs regimens: experience from the PRACTECAL-PKPD study. Nyang'wa, Bern-Thomas, Dr; Moore, David, Prof; Davies, Gerraint, Prof; Kloprogge, Frank, Dr; TB Science 2019 at the 50th UNION World Conference on Lung Health, October 2019, Hyderabad.

[Oral presentation conference abstract in Appendix]

6. TBS-02-04 PRACTECAL-VAMS: a successful novel approach to microsampling to determine TB drugs levels. M. Zimmerman, I. Motta, V. Dartois, C. Berry, R. Moodliar, B.-T. Nyang'wa for the TB-PRACTECAL Study Group. TB Science 2021 at the 52nd UNION World Conference on Lung Health, October 2021.

Related peer-reviewed publications co-authored during the PhD but not included in this thesis

7. Sedona Sweeney, Yoko V Laurence, Catherine Berry, Maninder Pal Singh, Matthew Dodd, Katherine Fielding, Emil Kazounis, Ronelle Moodliar, Varvara Solodovnikova, Zinaida Tigay, Irina Liverko, Nargiza Parpieva, Ilhomjon Butabekov, Ruzilya Usmanova, Mohammed Rassool, Ilaria Motta, George Mokua Nyangweso, Pascal Jolivet, Tleubergen Abdrasuliev, Soe Moe, Pei Sun Aw, Nazgul Samieva, Bern-Thomas Nyang'wa, 24-week, all-oral regimens for pulmonary rifampicin-resistant tuberculosis in TB-PRACTECAL trial sites: an economic evaluation, The Lancet Global Health, Volume 13, Issue 2, 2025, Pages e355-e363, ISSN 2214-109X, https://doi.org/10.1016/S2214-109X(24)00467-4.

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1.7. PhD related conference talks and presentations

[not included in thesis]

1. Pregnancy outcomes for patients treated with new and repurposed drugs for drug-resistant tuberculosis. AS-UnionConf-2022-00623. Nathalie Lachenal, Catherine Hewison, Catherine Berry, Carole Mitnick, Saman Ahmed, Elna Osso, Mathieu Bastard, Sylvine Coutisson, Emil Kazounis, Ilaria Motta, Bern-Thomas Nyang'wa. Oral abstract presentation at the Union World Conference on Lung Health, 8-11 November 2022.

2. Efficacy and safety results in participants co-infected with HIV from TB-PRACTECAL Clinical Trial. I. Motta, C. Berry, E. Kazounis, M. Dodd, K. Fielding, B.-T. Nyang'wa, TB-PRACTECAL team. Oral abstract session (A-AIDS-2022-01572) at AIDS 2022, the 24th International AIDS Conference, Montreal, Canada, from 29 July to 2 August 2022.

3. TB-PRACTECAL RESULTS: 24 WEEK ALL-ORAL REGIMENS FOR RIFAMPICIN RESISTANT TUBERCULOSIS. Bern-Thomas Nyang'wa, Emil Kazounis, Ilaria Motta, Matthew Dodd, Katherine Fielding, Catherine Berry, for TB-PRACTECAL team. Oral late breaker presentation at the Conference on Retroviruses and Opportunist infections(CROI2022). https://ww2.aievolution.com/cro2201/index.cfm?do=abs.viewAbs&abs=3299

4. SP-34 TB-PRACTECAL: trial results and next steps. Stage 2 trial efficacy results, B. Nyang'wa. 52nd UNION World Conference on Lung Health, October 2021. https://theunion.org/sites/default/files/2021-10/UNION2021_Abstracts_High.pdf

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7. Are humanitarian organisations capable of implementing complex clinical trials? Key insights from a Phase II/III MDR-TB drug trial designed to produce registration standard data (TB-PRACTECAL-NCT02589782). Emil Kazounis and Bern-Thomas Nyang'wa. 51st UNION World Conference on Lung Health, October 2020.

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1.8. Structure of the thesis

Chapter 1: General introduction

Chapter 2: TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II–III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis. [paper 1]

A 24 week all oral regimen for rifampicin resistant tuberculosis. [paper 2]

Short oral regimens for pulmonary rifampicin-resistant tuberculosis (TB-PRACTECAL): an open-label, randomised, controlled, phase 2B-3, multi-arm, multicentre, non-inferiority trial [paper 3]

Chapter 3: Additional population pharmacokinetics and pharmacodynamics methods.

Population pharmacokinetics and pharmacodynamics of investigational regimens' drugs in the TB-PRACTECAL clinical trial (the PRACTECAL-PKPD study): a prospective nested study protocol in a randomised controlled trial. [paper 4]

Chapter 4: Linezolid population pharmacokinetics and probability target attainment

Chapter 5: Pretomanid population pharmacokinetics and probability target attainment

Chapter 6: Clofazimine population pharmacokinetics and probability target attainment

Chapter 7: Bedaquiline population pharmacokinetics and probability target attainment

Chapter 8: Discussion and conclusion

Appendix

Chapter 2: The TB-PRACTECAL randomised controlled trial.

2.1. Introduction

This chapter consists of the methods for the tuberculosis pragmatic clinical trial for a more effective, concise, and less toxic regimen (TB-PRACTECAL) in Paper 1, the interim results of the randomised controlled clinical trial in Paper 2 and the final results in Paper 3.

The first patient was randomised in January 2017. Recruitment was terminated early following recommendations from both the independent data and safety monitoring board and the independent Scientific Advisory Committee. A total of 552 patients, 75% of planned sample size, were randomised to one of the four arms of whom 301 (54.5%) were in the stage 2 arms (SoC or BPaLM). A greater than expected number of trial participants were enrolled into arms that were to be discontinued for stage 2 (BPaLC and BPaL arms), due to slower transition to stage 2 than planned caused by the COVID pandemic so the results of these regimens are presented here as well.

Results based on an interim data lock of patients followed up until 18th March 2021 (end of randomisation date) are presented in Paper 2. The modified intention to treat (mITT) populations for SoC and BPaLM were 66 and 62 participants respectively. BPaLM was superior to SoC with an unfavourable outcome proportion of 19% and 48% respectively.

After the last patient reached 72 weeks post randomisation, in September 2022, the final database lock and analysis of TB-PRACTECAL data was performed. In this final analysis, there are 143 and 138 participants in the mITT for BPaLM and SoC. There are also 126 and 122 participants from BPaLC and BPaL arms respectively. The final results in table 2.1 reconfirm the non-inferiority and superiority of the BPaLM arm when compared to the SoC arm in a randomised and controlled trial design with increased precision. The BPaLC and BPaL arms are also each non-inferior and superior to the SoC.

Table 2.1 – Final primary outcomes for TB-PRACTECAL in the mITT population at 72 weeks post randomisation.

	SoC	BPaLM	BPaLC	BPaL
Number in mITT population	137	138	115	111
Number with no unfavourable outcome¥	81 (59.1%)	121 (88.3%)	88 (76.5%)	96 (86.5%)
Number with an unfavourable outcome	56 (40.9%)	16 (11.7%)	27 (23.5%)	15 (13.5%)
Unadjusted risk difference (two-sided 96.6% / 95%* confidence interval)	-	-29.2% (-39.8% to -18.6%)	-17.4% (-28.7% to -6.1%)*	-27.4% (-37.8% to -17.0%)*
Deaths	5 (3.7%)	0 (0%)	1 (0.9%)	1 (0.9%)
Early discontinuations	50 (36.5%)	11 (8.0%)	11 (9.6%)	11 (9.9%)
Treatment failure	0 (0%)	0 (0%)	1 (0.9%)	0 (0%)
Lost to follow-up at 72 weeks	1 (0.7%)	1 (0.7%)	6 (5.2%)	0 (0%)
Withdrew consent after treatment completion	0 (0%)	3 (2.2%)	3 (2.6%)	0 (0%)
Recurrence	0 (0%)	1 (0.7%)	5 (4.4%)	3 (2.7%)

¥ unfavourable outcome consists of death, treatment failure, treatment discontinuation, loss to follow-up, and recurrence.

2.2. Paper 1 – methods for the TB-PRACTECAL clinical trial

Berry C, du Cros P, Fielding K, Gajewski S, Kazounis E, McHugh TD, Merle C, Motta I, Moore DAJ, **Nyang'wa BT**. TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II-III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis. Trials. 2022 Jun 13;23(1):484. doi: 10.1186/s13063-022-06331-8.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1700547	**Title**	Dr
First Name(s)	Bern-Thomas		
Surname/Family Name	Nyang'wa		
Thesis Title	Short, effective and safe all-oral treatment for rifampicin resistant tuberculosis: TB-PRACTECAL trial and its drugs pharmacokinetics		
Primary Supervisor	Prof. David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMC Trials		
When was the work published?	June 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the Chief Investigator of this large multicentre, randomised controlled trial. I conceived the study. I chaired the protocol writing committee which developed the protocol. I drafted the first version of the manuscript together with the first author, reviewed further iterations and approved the final version.

SECTION E

Student Signature	[redacted]
Date	Jun 17, 2024

Supervisor Signature	[redacted]
Date	Jun 17, 2024

Trials

STUDY PROTOCOL Open Access

TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II–III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis

Catherine Berry[1*], Philipp du Cros[1,2], Katherine Fielding[3], Suzanne Gajewski[4], Emil Kazounis[1], Timothy D. McHugh[5], Corinne Merle[6], Ilaria Motta[1], David A. J. Moore[7] and Bern-Thomas Nyang'wa[8]

Abstract

Background: Globally rifampicin-resistant tuberculosis disease affects around 460,000 people each year. Currently recommended regimens are 9–24 months duration, have poor efficacy and carry significant toxicity. A shorter, less toxic and more efficacious regimen would improve outcomes for people with rifampicin-resistant tuberculosis.

Methods: TB-PRACTECAL is an open-label, randomised, controlled, phase II/III non-inferiority trial evaluating the safety and efficacy of 24-week regimens containing bedaquiline and pretomanid to treat rifampicin-resistant tuberculosis. Conducted in Uzbekistan, South Africa and Belarus, patients aged 15 and above with rifampicin-resistant pulmonary tuberculosis and requiring a new course of therapy were eligible for inclusion irrespective of HIV status. In the first stage, equivalent to a phase IIB trial, patients were randomly assigned one of four regimens, stratified by site. Investigational regimens include oral bedaquiline, pretomanid and linezolid. Additionally, two of the regimens also included moxifloxacin (arm 1) and clofazimine (arm 2) respectively. Treatment was administered under direct observation for 24 weeks in investigational arms and 36 to 96 weeks in the standard of care arm. The second stage of the study was equivalent to a phase III trial, investigating the safety and efficacy of the most promising regimen/s. The primary outcome was the percentage of unfavourable outcomes at 72 weeks post-randomisation. This was a composite of early treatment discontinuation, treatment failure, recurrence, lost-to-follow-up and death. The study is being conducted in accordance with ICH-GCP and full ethical approval was obtained from Médecins sans Frontières ethical review board, London School of Hygiene and Tropical Medicine ethical review board as well as ERBs and regulatory authorities at each site.

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Discussion: TB-PRACTECAL is an ambitious trial using adaptive design to accelerate regimen assessment and bring novel treatments that are effective and safe to patients quicker. The trial took a patient-centred approach, adapting to best practice guidelines throughout recruitment. The implementation faced significant challenges from the COVID-19 pandemic. The trial was terminated early for efficacy on the advice of the DSMB and will report on data collected up to the end of recruitment and, additionally, the planned final analysis at 72 weeks after the end of recruitment.

Trial registration: Clinicaltrials.gov NCT02589782. Registered on 28 October 2015.

Keywords: Multidrug-resistant tuberculosis, Bedaquiline, Linezolid, Clofazimine, Pretomanid, Moxifloxacin, Clinical trial, Phase 2/3, Multiarm multistage, RCT

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	A randomised, controlled, open-label, phase II–III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis (TB-PRACTECAL)
Trial registration {2a and 2b}.	Clinicaltrials.gov registration number NCT02589782
Protocol version {3}	Version 7.0 of 13 July 2020 (South Africa, inclusion criteria ≥ 15 years) Version 7.1 of 14 August 2020 (Belarus and Uzbekistan, inclusion criteria ≥18 years)
Funding {4}	Médecins sans Frontières
Author details {5a}	• Médecins Sans Frontières • London School of Hygiene and Tropical Medicine, London, UK • University College London, London, UK • Swiss Tropical and Public Health Institute • Burnet Institute, Melbourne, Australia • The Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization.
Name and contact information for the trial sponsor {5b}	Bern-Thomas Nyang'wa, Plantage Middenlaan 14, 1018 DD, Amsterdam, The Netherlands Bern.nyangwa@london.msf.org +44(0) 788 580 4202
Role of sponsor {5c}	Médecins sans Frontières as sponsor, is responsible for the design, collection, trial management and has final authority over submission. Data analysis will be performed by LSHTM.

Introduction

Background and rationale {6a}

The emergence of rifampicin-resistant tuberculosis (RR-TB), defined as TB caused by strains of *Mycobacterium tuberculosis* (MTB) resistant to at least rifampicin (R), has complicated global efforts to control the TB epidemic. Approximately half a million cases of RR-TB occur in the world annually, representing about 6% of the world's annual TB burden. Currently, around 38% of people with RR-TB are initiated on treatment and there is an urgent need to scale up treatment programmes [1]. Scale-up is being severely hampered by financial, political, logistical, and technical obstacles and one of the most important challenges is the current standard of care (SOC) [2]. The study was initially conceived as targeting multidrug-resistant tuberculosis (MDR-TB) which indicates additional resistance to isoniazid (H); however, as current treatments for MDR-TB and RR-TB are the same, the two terms in this protocol can be considered interchangeably. Additionally, since this study was conceived, the definition of extensively drug-resistant TB has been updated and is now known as pre-XDR [3]. The protocol refers to the pre-2021 definition of XDR.

The current treatment regimen used to treat RR-TB has poor efficacy. In a recent individual patient meta-analysis of treatment outcomes for pulmonary RR TB, only 61% of patients had successful outcomes, whilst 16% were lost to follow up and 14% died [4]. This poor effectiveness combined with high costs and implementation challenges, prevents many national TB programmes from offering treatment for MDR-TB [5]. This in turn fuels the spread of RR-TB infections [6]. There is clearly a global need for an improved treatment regimen for RR-TB that is efficacious, safe, tolerable, and that can be implemented quickly in a variety of geographic, epidemiologic, and programmatic settings. Given the high rates of HIV co-infection among certain populations of patients with RR-TB [7], it is imperative that patients with HIV be included in any new treatment regimen strategies.

Recently, several new anti-tuberculosis agents have been developed or re-purposed, including bedaquiline (TMC207; B), delamanid (OPC-67683, D), pretomanid

(PA-824; Pa) and the oxazolidinones, including linezolid (Lzd). These agents each act upon a completely novel target in the tuberculosis bacillus and have the potential to prove highly effective, especially when combined with one another and with existing antituberculosis drugs. In addition, there is promising evidence from phase II clinical trials for some of these new drugs when used with existing anti-tuberculosis drugs [8, 9]. Shortened treatment regimens have been explored in phase III trials using existing antituberculosis medications in novel combinations (STREAM study) [10] and in several ongoing trials [11].

TB-PRACTECAL is evaluating novel combinations of new and existing antituberculosis drugs in a 6-month, all-oral treatment regimen for safety and efficacy outcomes. Regimens have been selected for their potential efficacy, safety and ease of implementation in the field.

Objectives {7}

Primary objectives

Stage 1

Identify regimens containing bedaquiline and pretomanid for further evaluation based on safety and efficacy outcomes after 8 weeks of treatment.

Stage 2

Evaluate the safety and efficacy of the investigational regimens containing bedaquiline and pretomanid compared with the SOC at 72 weeks post-randomisation.

Secondary objectives

Stage 1

1. To compare the frequency of serious adverse events (SAE), and grade 3 and higher adverse events (AE's).

Stage 2

1) To compare the rates of culture conversion in liquid media between the SOC and investigational arms at specified time periods after randomisation (i.e. 12 weeks, 24 weeks);
2) To compare the frequency of SAEs, grade 3 and higher AEs between the SOC arm and investigational arms; and
3) To compare unfavourable outcomes between the SOC arm and investigational arms (including failure, treatment discontinuation, death, loss to follow-up, still on treatment at 108 weeks and recurrence) at specified time periods post randomisation (i.e. 24 weeks, 48 weeks and 108 weeks).

Trial design {8}

This is a multi-centre, open-label, multi-arm, randomised, controlled, phase II-III trial; evaluating short treatment regimens containing bedaquiline and pretomanid in combination with existing and re-purposed anti-TB drugs for the treatment of biologically confirmed pulmonary multidrug-resistant TB (MDR-TB).

The study is divided into two stages, with a seamless transition between the stages, meaning recruitment into an arm will only stop after a decision has been taken following stage 1 primary endpoint data analysis. Each randomised patient will complete his/her allocated treatment unless an unfavourable endpoint is reached. All recruited patients will be followed up for 108 weeks post randomisation unless they die, withdraw consent earlier or are censored at no earlier than 72 weeks. The locally approved SOC regimen for MDR-TB is used as the internal control for both safety and efficacy.

The first stage corresponds to a Phase II trial of safety and preliminary efficacy in patients with MDR-TB. Patients are recruited into 3 parallel bedaquiline (B) and pretomanid (Pa) containing regimen arms plus a SOC control. The main objective of stage 1 is to select drug regimens for evaluation in stage 2 based on 8-week safety and efficacy endpoints. All stage 1 patients undergo intensive cardiological evaluations to establish the early QT-specific liability of the regimens, and also closely monitor for early haematological and hepatic events.

Investigational arms that do not meet predefined safety and efficacy criteria (percent of culture conversion > 40%; percent of unfavourable outcomes <45%) are not considered for further evaluation. The arms that meet these pre-defined safety and/or efficacy criteria will be eligible to be evaluated for long-term safety, tolerability and efficacy in stage 2.

If fewer than two arms are available for stage two assessments, the Scientific Advisory Committee (SAC) makes recommendations on whether new arms should be introduced in the study. If more than two investigational arms are available for the stage 2 assessment, the SAC makes recommendations on which two arms are eligible to be taken forward to the trial steering committee.

The second stage corresponds to a phase III trial. Patients in this stage were to be recruited into up to 2 arms chosen from stage 1 plus the SOC. The regimens are primarily evaluated for efficacy in comparison with the SOC arm at 72 weeks post-randomisation. The primary efficacy outcome in stage 2 is a composite endpoint of the percentage of unfavourable outcomes (see section 7.1 for outcome definitions). Secondary outcomes include safety outcomes, and in particular, the percentage of patients experiencing SAEs and/or Grade 3 or 4 AEs during the treatment.

Stage 1 patients enrolled in arms that are continued to stage 2 are included in the sample size for stage 2. After the last enrolled patient has reached 72 weeks, all patients who have not reached their secondary endpoint are to be censored.

Methods: Participants, interventions and outcomes

Study setting {9}

The study is conducted in seven trial sites, in three countries. In Uzbekistan, the trial is implemented in four rayons of Karakalpakstan and Tashkent city. In Karakalpakstan, each of these rayons has a central clinic and several directly observed therapy (DOT) corners where trial patients get ambulatory care. Hospitalisation of trial participants (for severely ill patients or per local procedures) is in the Republican Specialised Scientific-Practical Medical Centre for Phyisiology and Pulmonology hospital in Tashkent City or Nukus TB2 hospital in Karakalpakstan. In Kwa-Zulu Natal province of South Africa, patients are hospitalised in Doris Goodwin, Don McKenzie and King Dinuzulu Hospitals, and in Gauteng province, the trial is conducted in Helen Joseph Hospital. In Belarus, the trial is conducted in Minsk City and Oblast. Participants are primarily followed up and hospitalised at the Republican Scientific and Practical Centre for Pulmonology and Tuberculosis hospital.

Eligibility criteria {10}

Inclusion criteria

Patients eligible for inclusion in the trial fulfiled all of the following criteria:

- Male or female patients aged 15 years or above (where locally approved), regardless of HIV status;
- Microbiological test (molecular or phenotypic) confirming the presence of *M. tuberculosis* in sputum;
- Resistant to at least rifampicin by either molecular or phenotypic drug susceptibility test; and
- Completed informed consent form (ICF).

Exclusion criteria

Patients were not eligible for inclusion in the trial if they meet any of the following criteria:

- Known allergies, hypersensitivity, or intolerance to any of the study drugs;
- Pregnant, breast-feeding, or unwilling to use appropriate contraceptive measures if of childbearing potential;
- Alanine transaminase (ALT) and/or aspartate transaminase (AST) and/or bilirubin >3 times the upper limit of normal;
- Taking any medications contraindicated with the medicines in the trial;
- Fredericia corrected QT interval (QTcF) > 450 ms;
- One or more risk factors for QTc prolongation (excluding age and gender) or other uncorrected risk factors for torsades de pointes (TdP);
- History of cardiac disease, syncopal episodes, symptomatic or significant asymptomatic arrhythmias (with the exception of sinus arrhythmia);
- Any baseline laboratory value consistent with Grade 4 toxicity;
- Moribund;
- Known resistance to bedaquiline, pretomanid, linezolid or delamanid;
- Any other condition (social or medical) which, in the opinion of the investigator, would make study participation unsafe;
- Prior use of bedaquiline and/or pretomanid and/or linezolid and/or delamanid for one or more months;
- Patients not eligible to start a new course of MDR-TB/ XDR TB treatment according to local protocol, including but not limited to:

  a) currently on MDR-TB treatment for at least 2 weeks (and not failing),
  b) no permanent physical address,
  c) loss to follow-up in previous treatment with no change in circumstance and motivation.

- Tuberculous meningoencephalitis, brain abscess, osteomyelitis or arthritis.

Who will take informed consent? {26a}

An ICF in clear, simple language is provided to the patient. The investigator collects written consent from each patient before any study-specific procedure is conducted. Two original ICFs are completed, dated and signed personally by the patient and by the investigator. The patient is given one signed original form; the second original is kept by the investigator.

If the patient is unable to read, a relative or an impartial witness is present during the informed consent discussion. The patient gives consent orally and, if capable of doing so, completes, signs (or thumbprints) and personally dates the information and consent form. The witness then completes, signs and dates the form together with the investigator.

For individuals under the legal adult age, both the patient and legal guardian must fully understand and agree to participate. An assent is signed by the patient as well as an ICF by the legal guardian prior to screening.

All ICF documents and supporting patient materials are approved by the local ethics committee.

Table 1 Standard of care drugs and dosing

Drug	Recommended dose by weight							
	30–35 kg	36–40 kg	41–45 kg	46–50 kg	51–55 kg	56–60 kg	61–70 kg	>70 kg
Isoniazid (high dose)	By weight, 15 mg/kg. Max 600 mg							
Ethambutol	800mg	800mg	800mg	800mg	1200mg	1200mg	1200mg	1200mg
Pyrazinamide (20–30 mg/kg) Max 2000 mg	800 mg	800 mg	1200 mg	1200 mg	1600 mg	1600 mg	1600 mg	2000 mg
Amikacin	500 mg	500 mg	750 mg	750 mg	1000 mg	1000 mg	1000 mg	1000 mg
Levofloxacin	750 mg	750 mg	750 mg	750 mg	1000 mg	1000 mg	1000 mg	1000 mg
Moxifloxacin	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg
Ethionamide/prothionamide	500 mg	500 mg	500 mg	500 mg	750 mg	750 mg	750 mg	750 mg
Terizidone/cycloserine	By weight (15–20 mg/kg)	750 mg	750 mg	750 mg	750 mg	750 mg	750 mg	750 mg
Para-aminosalicylic acid	4 g	8 g	8 g	8 g	8 g	8 g	8 g	8 g
Clofazimine	100 mg							
Linezolid	300 mg	600 mg	600 mg	600 mg	600 mg	600 mg	600 mg	600 mg
Bedaquiline	400mg once daily for 2 weeks then 200mg three times a week							
Delamanid	100 mg twice daily							
Imipenem/cilastatin	1000 mg imipenem/1000 mg cilastatin every 12 h							
Amoxicillin/clavulanate	500/125mg twice daily (*ONLY* for use in combination with Imipenem / cilastatin, give orally 30min before infusion)							

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Separate consent procedures and forms are used for participation in the trial sub-studies [12–14].

Interventions

Explanation for the choice of comparators {6b}

The comparator is the locally approved SOC which is as much as possible consistent with the WHO recommendations for the treatment of RR-TB. The regimen chosen varies depending on the country as well as over time to ensure those randomised to this regimen could access the best available care. For longer regimens, treatment is individualised with the constituent drugs changing depending on the proven or expected drug susceptibility testing (DST) of the infecting bacilli. The algorithm is described in the country-specific clinical guidelines, implemented alongside protocol v 7.0/7.1 and includes the use of at least four drugs including bedaquiline (B), a later-generation quinolone - moxifloxacin (Mfx) or levofloxacin (Lfx), linezolid (Lzd), clofazimine (Cfz), pyrazinamide (Z), prothionamide/ ethionamide (Pto/Eto) or cycloserine (Cs)/ terizidone (Trd). Other drugs such as amikacin, ethambutol (E), high-dose isoniazid, delamanid, para-aminosalicylic acid (PAS), imipenem/cilastatin and meropenem may also be used. A standardised shorter regimen or modified shorter regimen for RR-TB patients with no second-line drug resistance may be used if approved locally (Table 1).

Intervention description {11a}

Investigational regimens in stage 1:

Regimen 1: bedaquiline + pretomanid + linezolid + moxifloxacin for 24 weeks
Regimen 2: bedaquiline + pretomanid + linezolid + clofazimine for 24 weeks
Regimen 3: bedaquiline + pretomanid + linezolid for 24 weeks

Investigational regimen in stage 2 (Table 2):
Regimen 1: bedaquiline (B) + pretomanid (Pa) + linezolid (Lzd) + moxifloxacin (Mfx) for 24 weeks

Criteria for discontinuing or modifying allocated interventions {11b}

Treatment interruptions

Patients may interrupt/pause treatment for up to 14 consecutive days and be able to restart. This may result from the investigator temporarily withholding the treatment due to an adverse event or other social/logistical reasons. After sufficient recovery and strictly in line with the current version of the TB-PRACTECAL Clinical Guidelines, the patient may be restarted on the same treatment following consultation with the medical monitor.

Patients may also miss treatment due to challenges with adherence. The investigator and trial team support

Table 2 Investigational regimen drugs and dosing

Bedaquiline	400 mg once daily for 2 weeks followed by 200 mg 3 times per week for 22 weeks
Pretomanid	200mg once daily for 24 weeks
Moxifloxacin	400 mg once daily for 24 weeks
Linezolid	600mg daily for 16 weeks then 300mg daily for the remaining 8 weeks (or earlier when moderately tolerated)
Clofazimine	50 mg (less than 33 kg), 100 mg (more than 33 kg) for 24 weeks

the patient in identifying any underlying causes. Up to 14 consecutive days can be missed and treatment recommenced. If the patient misses greater than 14 consecutive days or is adjudged to have poor adherence as defined in TB-PRACTECAL Clinical Guidelines, they should permanently discontinue treatment. If treatment discontinuation is the final outcome, the investigator, with the support of the medical monitor, is responsible for linking the patient to further care.

Patients missing some days during the treatment phase should extend the treatment phase by the number of days missed. In this case, the last visit of the treatment period should be delayed to the date of the last dose.

Discontinuation and withdrawal criteria

Patients must discontinue study treatment, whatever trial regimen they have been allocated to, with any of the following events:

- Grade 3 or higher QT prolongation and other cardiac rhythm disturbances
- Grade 3 or higher hearing loss
- Patients who are felt to be non-adherent by the Investigator as evidenced by missing more than 2 consecutive weeks of treatment or meeting criteria outlined in the Clinical Guidelines.
- Patients who withdraw consent
- Permanently stopping or adding at least one drug in an investigational arm or two drugs in the SOC. Dose reduction or short holidays of less than 2 weeks will not be considered as significant modifications. Restarting treatment should only be done with the explicit recommendation from the Medical Monitor.
- At the discretion of the Investigator, a patient may discontinue treatment in case of any adverse event, laboratory abnormality, or intercurrent illness which, in the judgement of the Investigator, presents a substantial clinical risk to the subject with continued study regimens use.

If a patient's study regimen must be discontinued before the end of the treatment regimen, this will not result in automatic withdrawal of the patient from the study. Patients who discontinue treatment will be followed up to week 108, guided by the investigational schedule, unless they withdraw consent.

The management of patients who become pregnant whilst taking study drugs varies by site. In Belarus and Uzbekistan, patients who become pregnant and wish to continue their pregnancy are discontinued from the trial and are offered a regimen in line with national guidelines. In South Africa, patients and investigators are able to make individualized decisions in conjunction with the medical monitor whether to continue on the study regimen. All pregnancies are reportable to pharmacovigilance.

Strategies to improve adherence to interventions {11c}

All study treatments are delivered either through directly observed therapy (DOT) or video observed therapy (VOT). Treatment is delivered under direct observation by treatment supporters or nurses in health facilities, in patient homes or other community settings convenient to patients. Treatment is administered and observed daily 7 days a week in the investigational arms and at least 6 days a week in the SOC. The responsible study nurse or treatment supporter will be in charge of receiving the study drugs from the trial pharmacist, checking that patients receive the correct regimen and documentation of observed drug intake. Data on adherence and pill intake will be recorded on standardised forms and in the electronic case report form (eCRF).

Counselling and social support tailored to site needs as well as timely identification and management of adverse events are also key adherence support activities mandated by the sponsor.

Relevant concomitant care permitted or prohibited during the trial {11d}

All therapies (prescriptions or over-the-counter medications, including vitamins and herbal supplements) different from the trial drugs are recorded in the concomitant therapy section of the eCRF.

Prohibited drugs/absolute contraindications

The following therapies are not allowed during the trial: efavirenz; drugs known to significantly prolong the QTc interval, including neuroleptics-phenothiazines,

quinoline antimalarials, anti-arrhythmic drugs and fluoroquinolones other than those included in the trial regimens; drugs that may induce muscle damage such as HMG-CoA reductase inhibitors; strong CYP3A4 inducers; strong CYP3A4 inhibitors for more than 2 weeks; mono-amine oxidase inhibitors; drugs known to induce myelosuppression. Should any of the above-listed medication be administered concomitantly to study drugs, this is considered a protocol deviation.

Relative contraindicated medications

The following drugs have either established or suspected interactions or overlapping toxicities with the trial drugs. Therefore, their use should only be considered in situations where alternative options are either not available or are riskier than the administration of these drugs. Closer follow-up of patients taking these drugs is recommended. Site principal investigators should consider consulting the Sponsor Medical Monitor before prescribing them. Relatively contraindicated medications include antiretroviral medications, such as protease inhibitors, zidovudine and abacavir, selective serotonin reuptake inhibitors, tricyclic antidepressants and drugs known to cause limited QTc prolongation e.g. metoclopramide.

Provisions for post-trial care {30}

Patients who discontinue study treatment for any reason except if lost to follow-up will be offered an alternative, individualized rescue treatment based on their clinical condition and the latest drug susceptibility testing results and in line with national recommendations of the country. The rescue regimen is at the discretion of the Investigator in accordance with local standards and may include registered drugs accessible only through the trial. Investigators are encouraged to discuss the management of these patients with the Medical Monitor. Patients may also elect to have rescue treatment through their local tuberculosis programme.

Patients who discontinue treatment are encouraged to complete visits as much as possible per the investigational schedule (including SOC) unless consent is withdrawn. Continue all safety investigations as much as possible per investigational schedule up to week 108 post-randomisation and document all findings in the patient's file.

Following the discontinuation visit, sputum submissions, HIV tests, viral load and CD4 counts are no longer required for trial purposes. However, if performed for ongoing clinical management then the results should be requested and documented in the patient's file. TB drugs prescribed to the patient as part of a rescue treatment regimen are not considered investigational medical product.

Outcomes {12}

Stage 1 primary outcomes

- Efficacy outcome: percentage of patients with culture conversion in liquid media at 8 weeks post-randomisation.
- Safety Outcome: percentage of patients with treatment discontinuation and death at 8 weeks post-randomisation.

Stage 1 secondary outcomes

- Percentage of patients with grade 3 or higher QTc prolongation within 8 weeks post-randomisation
- Percentage of patients experiencing at least one SAE within 8 weeks post-randomisation
- Percentage of patients experiencing at least one new Grade 3 or higher AE within 8 weeks post-randomisation

Stage 2 primary outcome

- Percentage of patients with an unfavourable outcome at 72 weeks post-randomisation.

Stage 2 Secondary outcomes

- Percentage of patients with culture conversion at 12 weeks post-randomisation
- Median time to culture conversion
- Percentage of patients with an unfavourable outcome at 24 weeks post-randomisation
- Percentage of patients with an unfavourable outcome at 108 weeks post-randomisation
- Percentage of patients with SAEs or new Grade 3 or higher AEs at the end of treatment (at 24 weeks in investigational arms and at 80+ weeks in SOC arm)
- Percentage of patients with SAEs or new Grade 3 or higher AEs at 72 weeks post-randomisation
- Percentage of patients with SAEs or new Grade 3 or higher AEs at 108 weeks post-randomisation
- Mean single change in QTcF at 24 weeks post-randomisation
- Percentage of patients experiencing recurrence by week 48 in investigational arms (Table 3)

Participant timeline {13}

The trial visits are divided into screening, inclusion, week 1–8 (stage 1 and stage 2 differing investigations), week 9–24 (investigational and SOC arms similar

Table 3 Study outcome definitions

Death: Death of a patient from all causes.
Treatment failure in standard of care arm: *Conventional MDR-TB regimen* The presence of a positive mycobacterial culture in MGIT liquid media in each of two separate specimens taken at least four weeks apart (+/− 2 weeks) from week 28 until week 108 *Shorter MDR-TB regimen* The presence of a positive mycobacterial culture in MGIT liquid media in each of two separate specimens taken at least four weeks apart from week 16 (+/− 2 weeks) or later
Treatment failure in investigational arms: The presence of a positive culture in MGIT liquid media in each of two separate specimens taken at least four weeks apart from week 16 (+/− 2 weeks) or later.
Lost-to-Follow-up: A patient who has missed his/her appointment after completing treatment and cannot be traced until the end of the expected follow-up period (108 weeks or at time of censure).
Treatment discontinuation: A decision by an investigator to discontinue treatment: 1) either due to the need to significantly modify the trial regimen for whatever reason, 2) or due to the patient missing some or all drugs regularly 3) or due to the patient missing all drugs for more than 2 consecutive weeks
Still on treatment: A subject who is still taking treatment for M/XDR-TB 108 weeks after starting but hasn't been declared as treatment failure.
Culture conversion: At least two consecutive negative sputum cultures taken 4 weeks apart (+/− 2 weeks). The date of the first negative culture will be considered the conversion date.
Recurrence : A subject who has completed treatment without being declared a failure and who has subsequently been diagnosed and require MDR-TB treatment (for whom there is evidence that the recurrence is due to an MDR or XDR TB strain)
Re-infection: A subject who has completed treatment without being declared a failure and who has subsequently been diagnosed and require MDR-TB treatment but for whom there is evidence that the recurrence is due to a different strain to the baseline specimen. If the strain is a DS strain the patient is subsequently non-assessable.
Relapse: A subject who has completed treatment without being declared a failure and who has subsequently been diagnosed and require MDR-TB treatment and for whom there is evidence that the recurrence is due to the same strain recorded in the baseline specimen.
Unfavourable outcome: A composite outcome comprising death, treatment failure, treatment discontinuation, loss to follow up, still on treatment at 108 weeks and recurrence.

investigations) and week 25–108 (investigational and SOC arms differing investigations). Different visit windows apply for the treatment and follow-up period as follows: +/− 1 day for visits in the first 2 weeks, +/− 3 days for weekly visits and +/− 7 days for 4 or 8 weekly visits. Day 0 is defined as the day of randomisation. The inclusion visit may be done on the same day or a day earlier. Study visits in the first two weeks will be based on the day treatment was actually started and subsequent weekly visits are defined as seven-day multiples from that point. Trial investigational schedule schematic for stage 1 is described in Additional file 1.

Sample size {14}

The analysis of stage 1 is based on test arms only and there is no comparison with the SOC arm. Therefore, the sample size is based on the number required to detect culture conversion < 40% and/or a percentage of treatment discontinuation for any cause and death >45% in an investigational arm.

With 60 participants in an investigational arm evaluable for treatment discontinuation, 29% [15] patients or fewer would need to discontinue, to have 80% power with a one-sided alpha=0.05 to reject the null hypothesis of a true underlying discontinuation rate of 45% (or greater). (Sample size determination for one proportion $\{u(\sqrt{[\pi(1-\pi)]} + v\sqrt{[\pi 0(1-\pi 0)]}\}2/(\pi-\pi 0)2$, u=1-power, v=two-sided significance level).

Similarly, if there are 29% or fewer discontinuations, there would be 43–60 patients remaining per arm to evaluate culture conversion. In this scenario, 55% (33/60)–58% (24/43) of the patients would need to have culture conversion to have 80% power with a relaxed one-sided alpha=0.075 to reject the null hypothesis of a true underlying conversion rate of 40% (or lower).

Analysis at stage 2 is based on a non-inferiority design to assess efficacy. Sample size calculations are based on this efficacy non-inferiority comparison of the composite primary outcome. In order to allow for both the adaptive nature of the design and the multiple comparisons with three possible arms, an alpha of 1.7% was used.

The underlying assumptions for these power calculations are based on the failure rates seen in patients receiving the control regimen at the time of original protocol writing. An analysis of loss to follow up (LTFU) over time suggested an additional 10% LTFU rate per 6 months of treatment after the first 6-8 months. These data were also supported by a large individual patient data meta-analysis of more than 9000 MDR-TB patients [3]. If assumed that the control and investigational regimens perform the same on all variables included in the composite efficacy other than LTFU, then the likely decrease in LTFU rate expected in the investigational arms due to the shorter length of treatment would lead to the investigational arm performing better overall. Although the primary outcome is efficacy at 72 weeks, the final sample size allows for adequate power to assess the secondary outcome of efficacy at 108 weeks.

Therefore, assuming a failure rate of 50% in the control arm and of 45% in the investigational arms, 181 patients per arm would be needed for a delta of 12% with approximately 85% power and a one-sided 98.3% confidence interval (to allow for both the adaptive nature of the design and the multiple comparisons of the three arms).

The delta of 12% was chosen following extensive consultation. The benefits of reducing treatment duration from 9-24 months to 6 months, reduced pill burden, and all oral nature of the investigational regimens have considerable advantages which would outweigh a possible increase in failure rate as reflected in the 12% non-inferiority margin. This delta is also comparable to contemporary ongoing MDR-TB clinical trials which have been approved by the US Food and Drug Administration (FDA) and local regulatory agencies [9].

Information available from patients recruited by the end of stage 1 suggested that the number excluded from the modified intention to treat (mITT) population is closer to 10% and therefore the recruitment target was increased to 201 per arm.

Recruitment {15}

Patients in the catchment areas with a molecular WHO-approved rapid diagnostic test (WRDT) showing rifampicin resistance were assessed for eligibility by investigators in liaison with local clinics. Patients with sputum cultures showing rifampicin resistance or who were not responding to their current treatment could also be referred. Patients fitting initial eligibility criteria were invited to counselling sessions and after full informed consent, could be included in the study.

A community engagement strategy was developed that described the overall objectives, implementation and monitoring of trial community engagement activities. From this, in consultation with local stakeholders, context-adapted community engagement plans were developed.

The aims of these plans were (i) to engage in a two-way dialogue to harness local knowledge and patient insights towards better trial preparation, recruitment and retention of participants and (ii) to build a positive foundation of understanding, acceptance, goodwill and support in order to identify and overcome barriers to participation. These plans laid the groundwork for the models of care to deliver patient-centred care and cement partnerships with local TB providers. These plans are continuously reviewed and updated in response to recruitment challenges.

Additionally, expansion of trial catchment areas and new trial sites were added when recruitment was slower than anticipated.

Assignment of interventions: allocation

Sequence generation {16a}

Treatment allocation was done using ratios of 1:1:1:1 in stage 1 and 1:1 in stage 2. Randomisation lists were produced by the trial statistician for each stage of the study, stratified by study site. For stage 1 randomisation, the "ralloc" package in Stata [16] was used to create randomisation lists for each site (with block sizes of 8).

In stage 2, the sequence was generated by proprietary software also used to undertake the randomisation [17]. In stage 2, varying block sizes of 4 and 6 were used.

Concealment mechanism {16b}

In stage 1, the code for each individual was provided in a secure manner to the sites in separate, opaque sealed envelopes and assigned to individuals in the order in which they were enrolled in the study. The sealed randomisation envelopes look identical and were kept in a separate room, in a locked cupboard with restricted access. Each envelope had a sequential number and contained the details of the regimen the patient would receive. The randomisation list was kept by the trial statistician.

Implementation {16c}

The allocation sequence was generated by the trial statistician and envelopes (stage 1) or by the randomisation system (stage 2) provided to the sites. Randomisation was undertaken according to the local SOP at the request of an investigator, once all screening and inclusion activities

were complete. Personnel in charge of the randomisation, as well as the witness, were not involved in direct patient care. In stage 1, delegated personnel were responsible for opening the next sequential envelope, documenting the treatment allocation and assigning the study number. In stage 2, the same procedure was followed except randomisation personnel used an online, self-service randomisation system to receive the treatment allocation in lieu of envelopes [17]. Randomisation personnel then notified the investigator of the allocation.

Assignment of interventions: blinding

Who will be blinded {17a}

TB-PRATECAL is an open-label trial; however, the laboratory personnel and centralised electrocardiogram (ECG) reviewers are blinded to treatment allocation.

Procedure for unblinding if needed {17b}

Not clinically applicable.

Data collection and management

Plans for assessment and collection of outcomes {18a}

All study data are first recorded in source documents before being transcribed in the eCRF [15]. Radiology, ophthalmology, and audiometry data are acquired and recorded by the sites in sponsor developed forms and interpreted locally. ECGs are transmitted by the sites to a central ECG laboratory to undergo quality checks and blinded central review and reporting. Laboratory data is recorded onto the quality forms contained in the mycobacteriology and safety quality manuals before being transcribed into the eCRF. Where a laboratory information system conforming to the Code of Federal Regulations Title 21, Part 11 (21 CFR Part 11) requirement is available, the data will be transmitted directly from the laboratory information system into the clinical database.

The designated source documents which are agreed between the sponsor and the investigators at each site are available at the trial site, to allow retrospective checks that source data have been accurately and completely transcribed into the eCRF.

Plans to promote participant retention and complete follow-up {18b}

Retention in care is within the scope of the community engagement plan through activities to build mutual trust and respect between study staff and participants. Along with home-based care (in Uzbekistan), DOT and VOT tools, adherence guidelines have been designed according to the site needs. Individual and group counselling is available for participants throughout treatment and follow-up. An individualised strategy to meet patient needs has been put in place in all sites (transportation to the facility, follow up through secure social media, convenient appointments, engagement of social supports in adherence). In the event of missed visits or challenges with treatment adherence are identified, the study team makes every effort to trace the patient.

Data management {19}

An eCRF was designed to record all the data collected as per the protocol. An eCRF is completed for each participant. The eCRF, together with all trial related forms and logs are produced by the sponsor. The eCRFs have been built using OpenClinica [15], a fully validated secure web-enabled software that conforms to 21 CFR Part 11 requirements.

The delegated investigator staff enter the data required by the protocol, but the Principal Investigator is responsible for assuring that the data entered into the eCRF are complete, accurate, and consistent with the source documents and that entry and updates are performed in a timely manner. Corrections and alterations of data on the eCRF or source documents must be made by the investigator or by the delegated person from his/her team, dated and signed. Changes to the eCRF are tracked electronically in the database audit trail.

Adverse events are coded using the Medical dictionary for regulatory activities (MedDRA) terminology [18]. Concomitant medications are coded using international non-proprietary names (INN) [19].

The Data Manager, or their delegate, reviews the eCRF data entered by investigator staff for completeness and accuracy. Edit checks are built into the eCRF and contain univariate checks on the eCRF including missing values in required fields, range checks and valid values among others. Electronic data queries stating the nature of the problem and requesting clarification are created for discrepancies and missing values and sent to the investigational site via the electronic data capture system. Details are documented in the TB-PRACTECAL Data Management Plan.

Once the trial data has been verified for completeness and accuracy, the database will be locked in compliance with the database locking standard operating procedure (SOP).

Confidentiality {27}

The Principal Investigator (or delegate) is responsible for recording the patient's personal details, screening number and unique trial number in the subjects' identification list. This list is kept in a lockable safe in the trial office, with access restricted to authorised trial staff only. All laboratory specimens, including stored specimens, as well as trial reports, data collection tools, and

administrative forms are only identified by using the patient's unique trial number. Names are not used on any of these documents. All local databases are secured with password-protected access systems. The Investigator ensures anonymity of the patient and that all documents are anonymised before being transmitted to the sponsor.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

WRDT testing will be used to screen for eligibility. Those participants with TB isolates resistant to rifampicin by the rapid molecular tests will then be evaluated by MGIT drug sensitivity testing (DST) for confirmation of MDR-TB. Liquid culture will be done using the MGIT 960 system [20]. Rapid testing will be done according to site-specific SOPs as detailed in the Mycobacteriology Laboratory Manual.

Two sputum samples (1 early morning and 1 coached spot expectoration sample) will be collected from trial participants at least once in a month during investigational arms' treatment and once every two months during follow-up. DSTs will be performed on pure cultures from specimens obtained at baseline, during treatment and follow-up period, using MGIT. Susceptibility to the following drugs will be tested at baseline and from week 16 onwards if culture positive: H, R, E, Z, S (streptomycin), Km (kanamycin), Cm (capreomycin), Mfx and/or Ofx (ofloxacin). Culture isolates at the same intervals as above will be stored for minimum inhibitory concentration (MIC) determination for B, Pa, Lzd, Cfz +/− Mfx when indicated.

Mycobacterial DNA will be stored at baseline (D0, D7 and at W4 if the D0 and D7 DNA samples are not available) from all patients. In patients who revert after culture conversion or develop recurrent TB during the follow-up period after the end of TB treatment, genotyping will be performed on paired *M. tuberculosis* positive isolates (originating from that patient), in order to differentiate relapse and reversion from re-infection. Isolate DNA for such testing will be stored at the site and shipped to approved testing centres according to site-specific SOPs. If exportation of biological material is not allowed, then genotyping may be performed on site.

Refer to the TB-PRACTECAL Mycobacteriology Laboratory Manual for details of the standard procedures for the key methodologies, quality control practices, interpretation of findings and standardised terminology. The laboratory team will be blinded to the trial arm of the participants at all times when processing the samples.

All specimens planned for further analysis in sub-studies are detailed in the sub-study protocol [14].

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Demographic and baseline characteristics of the randomised patients will be summarised by treatment arm. The distribution of categorical variables will be summarised by counts and percentages. Quantitative variables will be summarised using the mean and standard deviation (SD) or median and inter-quartile range (IQR), where appropriate, and the minimum and maximum and sample size of non-missing data. Any imbalances of baseline characteristics across treatment arms identified through examination of these summaries will be noted.

The outcome data will be analysed by multiple regression modelling, with appropriate generalised linear models used to examine the effect of the intervention. The effects reported will be adjusted differences in proportions with confidence intervals, with the adjustment being for site. All subgroup analyses will be specified a priori in the Statistical Analysis Plan (which will be approved by the Data safety and monitoring board (DSMB) before the end of stage 1) and carried out using formal tests for interaction included in the statistical models and assessed for statistical significance using likelihood ratio tests.

The primary analysis will be per-protocol (PP); where patients will be analysed based on the treatment they actually received rather than the one they were allocated to and given the non-inferiority trial design, an intention to treat (ITT) analysis will also to be conducted.

Interim analyses {21b}

Following completion of stage 1 recruitment, the primary and safety analyses will be provided to the DSMB. The DSMB would then make a recommendation to the SAC as described above. A further interim analysis was planned after 90 patients per arm were recruited into stage 2 of the trial. Stopping was to be considered if a difference between randomised arms of at least 3 standard deviations in the interim analysis of a major endpoint achieved and the results had the potential to impact clinical practice. The final decision would be taken by the Trial Steering Committee based upon a recommendation of the DSMB.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Subgroup analyses will be performed for the following variables: HIV status, trial site, cavitation on chest x-ray, resistance pattern, previous TB treatment, smear positivity, smoking status, age, sex and SARS-CoV-2 status. Interaction tests between treatment group and the subgroups listed above will be carried out on the

additive (i.e. risk difference) scale for the efficacy and safety primary outcomes only. Results for treatment efficacy and safety will be reported, stratified by the factors. Possible reasons for the interaction, such as clinical differences between sites, will be explored. All subgroup analyses will be performed on the ITT, mITT and PP populations.

Additionally, post hoc analyses not originally described in the protocol will be mentioned in the statistical analysis plan.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

For the primary composite outcome, it is assumed that no negative outcome was reached unless one was observed. For culture conversion, it is assumed no culture conversion had occurred if culture conversion was not observed.

A complete case analysis is planned with no imputation.

Plans to give access to the full protocol, participant-level data and statistical code {31c}

The full protocol and statistical analysis plan will be made available as appendices during the publication of the trial results.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The trial is governed by a Steering Committee (SC), an independent Scientific Advisory Committee (SAC), an independent DSMB and the Project Management Team (PMT). The SC's main responsibility is to provide strategic, political and operational oversight to the trial to ensure the objectives are effectively met within the time frame and resources allotted. The SC approves the protocol and is the decision body for any trial stoppage decisions. The SAC is a committee external and independent from all project collaborators that provides scientific advice to Médecins sans Frontières (MSF) regarding new MDR-TB regimen projects including TB-PRACTECAL. It advises the PMT on the relevance and scientific validity of the trial designs and their implementation. The SAC makes the recommendation on arms to take forward from stage 1 to stage 2. The PMT's responsibility is translating the project strategic direction and objectives set by the steering committee into a clinical trial that will achieve the intended outcomes. This entails making operational (technical, financial, and administrative) choices and running the day-to-day aspects of the trial.

Composition of the data monitoring committee, its role and reporting structure {21a}

The DSMB is independent of the sponsor and all project collaborators. It is governed by the DSMB charter which describes its purpose and terms of reference. It consists of a statistician (Chair), a drug development expert, an HIV expert, a TB clinical trials expert and an MDR-TB clinical expert. The overall responsibility of the DSMB is to protect the ethical and safety interests of subjects recruited into the PRACTECAL trial. The committee reviews the accumulating unblinded safety data after every 40 patients recruited to the study or every three months whichever occurs first and meet at least every 6 months. Depending on this evaluation, the DSMB will make recommendations to the SC concerning the continuation, modification, or termination of the study.

Adverse event reporting and harms {22}

Adverse Events recording applies to both investigational and control arms in the trial. AE recording began upon initiation of study treatment and continued until the patient's last study visit. All AEs are recorded in the AE section of the eCRF. AEs can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a trial participant. The investigator must also promptly review all results of assessments performed as part of the trial, such as laboratory assessment results, ECGs, vital sign monitoring, physical examinations, etc. and assess them for clinically relevant changes compared to baseline. Each AE is evaluated to determine the severity grade: Grade 1–4 as per the latest version of the MSF Severity grading scale [21], its duration (start and end dates or if continuing at the end-of-study visit), its relationship to the study treatment, action taken with respect to study treatment (treatment maintained, dose reduced, permanently discontinued, temporarily discontinued, not applicable), whether medication or therapy was taken/given in relation to the AE and whether it is a serious adverse event (SAE).

In the study, ICH-GCP definitions for SAE are applied [22]

An adverse event of special interest is one of scientific and medical concern specific to the investigational drug(s), for which on-going monitoring and rapid communication by the investigator to the sponsor is appropriate. Such events require further investigation in order to characterise and understand them. Based on signals observed from previous studies, several AEs of special interest were identified for this trial:

- All grade 4 AEs which are not SAEs
- Grade 3 QT interval prolongation
- Other grade 3 dysrhythmias
- Grade 3 liver enzyme abnormalities (transaminases and bilirubin)
- Any grade of pancreatitis
- Any grade of optic nerve disorder
- Grade 3 peripheral neuropathy
- Any grade of seizures and fainting
- Any grade cataract formation

Every SAE and AE of special interest (AESI) is reported by the investigator to the sponsor's pharmacovigilance (PV) unit within 24 h of learning of its occurrence. Recurrent episodes, complications, or progression of the initial SAE/AESI are reported as follow-up to the original episode within 24 h of the investigator receiving the follow-up information. Additionally, pregnancy, overdose and malignancy not otherwise serious, require expedited reporting using a similar process.

All adverse drug reactions that are both serious and unexpected are subject to expedited reporting to the National Regulatory Authorities (NRA) and ethics review boards (ERBs). The sponsor is responsible for reporting these events to NRA whilst the site principal investigator is responsible for reporting the events to the local ERB. In the context of this study, reporting to NRAs may be delegated to the sites with close support from the sponsor as detailed in the corresponding SOP.

Fatal or life-threatening suspected unexpected serious adverse drug reactions should be reported as soon as possible and no later than 7 calendar days after first knowledge by the sponsor of the case. Unexpected Serious ADRs that are not fatal or life-threatening must be notified as soon as possible and no later than 15 days after first knowledge by the sponsor of the case. Unless specifically requested by NRAs/ERBs, all SAEs, that are not considered as unexpected ADR are summarised in annual safety reports and submitted to NRA and ERB in due time.

Frequency and plans for auditing trial conduct {23}

Prior to study start, a Monitoring Plan and Monitoring SOP was developed, agreed upon between the external monitor and the PMT. The site principal investigator will allow the monitors to visit the site and facilities where the study will take place in order to verify compliance with the protocol requirements, ICH-GCP (International Council on Harmonisation – Good Clinical Practice) and WHO-GCLP (World Health Organization – Good Clinical Laboratory Practice). Training sessions on GCP, GCLP and on protocol implementation were organised for the investigators and all study staff prior to recruitment start and as staff join the project. Instruction manuals and SOP will be distributed to all the study centres.

Study monitoring is carried out at regular intervals, depending on the recruitment rate, to verify data quality and study integrity. At the end of each monitoring visit, and based on monitoring visit reports, the PMT will be responsible for controlling recruitment rates, ineligibility, non-compliance, protocol deviations and dropouts overall and in each study centre, completeness and timeliness of data and compliance with GCP, GCLP and applicable regulations

A final monitoring visit will be conducted at the end of the trial, after the last patient, last visit (LPLV), and once the database is locked.

In addition to the planned monitoring activities, the trial may be evaluated by external auditors appointed by the sponsor and by government inspectors who must be allowed access to CRFs, source documents, study files, and study facilities. This will be independent from investigators and sponsors.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

If the protocol must be altered after it has been signed, the modification or amendment must be discussed and approved by the Principal Investigators and the sponsor. The protocol amendment must be drafted and signed by both parties. All amendments are submitted to the relevant Ethics Committees and NRAs. Administrative amendments can be implemented immediately but amendments that affect other aspects can only be implemented after a favourable opinion of the Ethics Committee and NRA has been obtained and local regulatory requirements have been complied with. An amendment needed to eliminate immediate hazards to the participants in the study is exempted from this rule.

Dissemination plans {31a}

The results of the trial will be submitted for publication in an open-access peer-reviewed scientific journal and posted in a publicly accessible database of clinical study results within 12 months. Preliminary results will also be shared in global conferences. Communities involved in the study will be informed of the outcomes and other national or global stakeholders will receive relevant information.

Discussion

TB-PRACTECAL is a multi-arm, multi-stage clinical trial aimed at identifying safe and efficacious regimens to treat rifampicin-resistant tuberculosis. The adaptive trial design was chosen to assess a range of candidate

regimens and ensure seamless progression of the most promising regimen/s into phase III. The accelerated model was, if positive, designed to bring a shorter and more efficacious treatment to high-burden communities as soon as practicable. Current MDR/RR-TB treatment remains 9–20 months and carries a significant risk of adverse events. High-quality clinical research for MDR/RR-TB needs extensive resourcing and several years to be able to provide data given the ongoing need for extended follow up.

The trial takes an ambitious and pragmatic approach to regimen advancement compared with earlier explanatory trials into newer tuberculosis drugs such as bedaquiline and delamanid [8, 9, 23]. In doing so, a conservative safety approach is being taken with intensive oversight by the sponsor, regular monitoring from the independent DSMB and continuous pharmacovigilance.

The design features are notable for the continuously updated SOC which has changed radically in all centres since trial inception. This choice complicates the analysis however has aided in ongoing recruitment by ensuring those randomised to SOC will receive the best available treatment at any point in the trial. Patients enrolled in the stage 1 of the trial also continued their treatment arm through to week 108 and their findings will contribute to the stage 2 sample size.

Sites were selected based on a range of factors including differing geography, resource limitations, rates of second-line drug resistance and rates of HIV representing the diversity of contexts and sub-groups most affected by the RR-TB epidemic. Research experience is varied and so a supportive, risk-based monitoring approach was taken and tailored to site needs.

The study aims to add to the research base guiding the use of shorter MDR/RR-TB regimens composed by new and re-purposed drugs. During the study, encouraging results from uncontrolled NIX-TB clinical trial were published [24] and TB-PRACTECAL may complement these findings. Additionally, it may assist in answering whether an additional drug provides added benefit to BPaL regimens and will provide data on an alternative approach to linezolid dosing.

Limitations include limited generalizability to certain populations such as children under 15 and pregnant women who were excluded from entry into the trial. This was an open-label study and blinding was limited to laboratory staff. Outcome assessment was at the investigators discretion but had to be verifiable in the database and in line with the protocol. Any ambiguous outcomes were referred to an independent outcome adjudication committee for final classification. The safety approach meant that patients were discontinued from the trial under conservative rules which were based on the safety profile of the investigational regimens at the trial outset. This may not resemble routine care and limit the strength of the conclusions which can be drawn. However, all arms were handled under the same rules. The effectiveness of any candidate regimen should be further evaluated under programmatic conditions.

Trial status

The trial is currently operating under protocol version 7.0 or 7.1 (depending on site). The first patient was recruited on 16 January 2017.

Recruitment into stage 1 was completed in mid-2019. The transition procedures were followed per the protocol with all arms meeting the pre-specified eligibility criteria for stage 2. Following the recommendation from the Scientific Advisory Committee to proceed with investigational arms 1 and 2, the steering committee proposed to proceed to stage 2 with arm 1 only. The Sponsor accepted and implemented this decision. Transition from stage 1 to stage 2 was delayed with the COVID-19 pandemic and completed in late 2020. Randomisation into all 4 arms continued until transition was complete at each site.

The COVID-19 pandemic also impacted the trial sites to a varying extent. The Sponsor and sites collaborated to develop a mitigation plan. This allowed increased flexibility given limited patient movements but aimed to minimise impacts on data quality and patient safety. Ensuring continuity of care and treatment, managing infection control risks for staff and patients, and access to care for severe illness or adverse events were key priorities. An earlier switch to the less intensive investigation schedule for stage 2 (pre-dose ECG only, audiometry and slit lamp examinations as clinically indicated only), phone visits, accelerated implementation of VOT at every site and remote monitoring visits were some of the solutions put in place. Slow recruitment was another challenge caused by pandemic: some diagnostic facilities were closed, restriction in movements decreased number of screenings and TB diagnosis and other TB facilities were sometimes repurposed as COVID-19 wards.

In February 2021, the DSMB recommended that the steering committee terminate recruitment based on an observed difference in efficacy between study arms. This advice followed the DSMB charter procedures which recommended that stopping be considered if there was a difference between randomised arms of at least three standard deviations in the interim analysis of a major endpoint. The endpoint also needed to be one that would likely impact clinical practice.

The steering committee followed DSMB recommendations and recruitment ended on the 18th of March 2021.

All enrolled patients will continue to be followed up to at least week 72, post-randomisation.

TB-PRACTECAL plans to report data up to date of termination. A revised statistical analysis plan will be adapted for this analysis. The findings will be shared through conference presentations and via submission to a peer-reviewed journal. The trial will continue to follow and monitor the remaining patients through to last patient visit as planned and a final report will also be shared widely.

Abbreviations

ADR: Adverse drug reaction; AE: Adverse events; AESI: Adverse event of special interest; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; B: Bedaquiline; CD4: Cluster of differentiation 4 cells; CFR: Code of Federal Regulations; Cfz: Clofazimine; COVID-19: Coronavirus disease caused by SARS-CoV-2 virus; Cs: Cycloserine; DOT: Directly observed therapy; DS: Drug sensitive; DSMB: Data and Safety Monitoring Committee Board; E: Ethambutol; ECG: Electrocardiogram; eCRF: Electronic case report form; EDC: Electronic data capture; ERB: Institutional Ethics Review Board; Eto: Ethionamide; FDA: Food and drug administration; GLM: Generalised linear models; H: Isoniazid; HIV: Human immunodeficiency virus; ICF: Informed consent form; ICH-GCP: International Committee on Harmonisation of Good Clinical Practice; IMP: Investigational medicinal product; INN: International non-proprietary name; ITT: Intention to treat; IQR: Interquartile range; Lfx: Levofloxacin; LIS: Lab information system; LSHTM: London School of Hygiene and Tropical Medicine; Lzd: Linezolid; MDR: Multidrug-resistant; MedDRA: Medical Dictionary for Regulatory Activities; Mfx: Moxifloxacin; MGIT: Mycobacteria growth indicator tube; mITT: Modified intention to treat; MSF: Médecins Sans Frontières; MTB: *Mycobacterium tuberculosis*; NRA: National Regulatory Authority; Ofx: Ofloxacin; Pa: Pretomanid (PA-824); PAS: Para-amino-salicylate sodium; PMT: Project Management Team; PP: Per-protocol; Pto: Prothionamide; QT : Interval between Q and T waves on an ECG; QTc: QT interval corrected; QTcF: QT interval corrected using Fridericia's formula; R: Rifampicin; RR-TB: Rifampicin-resistant TB; SAC: Scientific Advisory Committee; SAE: Serious adverse events; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SC: Steering Committee; SD: Standard deviation; SOC: Standard of care; SOP: Standard operating procedure; SUSAR: Severe unexpected serious adverse drug reaction; TB : Tuberculosis; TdP: Torsades de Pointes; Trd: Terizidone; VOT: Video observed therapy; WHO: World Health Organization; XDR: Extensively drug-resistant; Z: Pyrazinamide.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-022-06331-8.

Additional file 1.

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Emilie Alirol, Aita Signorell, Elizabeth Allen, Stephen Murray, Kristen Lebeau and Jennifer Kealy assisted in drafting the original and/or revisions of the protocol. Matthew Dodd provided review of the statistical components of the manuscript. Emma Veitch edited the manuscript. Hannah Spencer assisted with revising the manuscript.

Authors' contributions {31b}

BN is the Chief Investigator; he conceived the study, led the proposal and protocol development. CB is the Global principal investigator, supported protocol implementation and lead the manuscript. IM is the medical monitor and supported protocol implementation and manuscript revision. CM, PdC, KF, TM and DM contributed to study design and to development of the proposal. KF is the lead trial statistician. TM is the lead trial microbiologist. EK is the clinical trial manager and implemented the protocol. SG is the lead external monitor and provided support with protocol revisions. All authors read and approved the final manuscript.

Funding {4}

Médecins sans Frontières funded the study and led the development of the study protocol and writing of the manuscript.

Availability of data and materials {29}

The trial data will be made available after the primary publication or twelve months after trial completion (whichever is earlier) upon reasonable request and in agreement with the MSF Data sharing policy.

Declarations

Ethics approval and consent to participate {24}

The study protocol, the participant Information and Consent Form (ICF), the eCRF, up-to-date versions of the Investigator Brochures or Summary of Product Characteristics (SmPC), as well as Principal Investigators qualifications has been submitted and approved by the following ethical boards:
Médecins Sans Frontières (MSF) Ethics Review Board
London School of Hygiene and Tropical Medicine (LSHTM) Ethics Committee
Uzbekistan National Ethics Committee
Ethics Review Committee of the Republican Scientific and Practical Centre for Pulmonology and Tuberculosis – Belarus
PharmaEthics Independent Ethics Committee in South Africa
University of Witwatersrand Human Research Ethics Committee in South Africa
The study did not start in any centre before written approval by these Ethics Committees had been obtained, the local regulatory requirements had been complied with, and the signature of the clinical study protocol of each contractual party involved had been obtained.
Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}

See annex.

Competing interests {28}

Philipp du Cros has received funding from TB Alliance for a project to analyse introduction and scale up of pretomanid and the NIX-TB regimen. Philipp du Cros is a member of the rGLC WPRO region. CM is currently staff members of the World Health Organization; the authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the WHONo other authors had conflicts to declare.

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Publisher's Note

2.3. Paper 2 – TB-PRACTECAL clinical trial interim results

Nyang'wa BT, Berry C, Kazounis E, Motta I, Parpieva N, Tigay Z, Solodovnikova V, Liverko I, Moodliar R, Dodd M, Ngubane N, Rassool M, McHugh TD, Spigelman M, Moore DAJ, Ritmeijer K, du Cros P, Fielding K; TB-PRACTECAL Study Collaborators. A 24-Week, All-Oral Regimen for Rifampin-Resistant Tuberculosis. N Engl J Med. 2022 Dec 22;387(25):2331-2343. doi:10.1056/NEJMoa2117166

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1700547	**Title**	Dr
First Name(s)	Bern-Thomas		
Surname/Family Name	Nyang'wa		
Thesis Title	Short, effective and safe all-oral treatment for rifampicin resistant tuberculosis: TB-PRACTECAL trial and its drugs pharmacokinetics		
Primary Supervisor	Prof. David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	The New England Journal of Medicine		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I have been the Chief Investigator and Project Manager of the TB-PRACTECAL trial. In this role, I led the conceptualisation of the TB-PRACTECAL randomised controlled trial, development of the trial protocol including chairing the protocol writing committee, oversaw the implementation of the protocol, data collection, data analysis, interpretation and results communication. I steered the research project and was the principal decision maker on the study implementation choices (site selection, clinical management guidance, data collection tools, quality assurance approaches etc) and analysis plan. I drafted together with some authors the first draft manuscript, communicated with the journal throughout revisions and approved the final manuscript

SECTION E

Student Signature	[redacted]
Date	Jun 17, 2024

Supervisor Signature	[redacted]
Date	Jun 17, 2024

ORIGINAL ARTICLE

A 24-Week, All-Oral Regimen for Rifampin-Resistant Tuberculosis

Bern-Thomas Nyang'wa, M.B., B.S., Catherine Berry, B.Med., Emil Kazounis, M.Med.Sci., Ilaria Motta, Ph.D., Nargiza Parpieva, Sc.D., Zinaida Tigay, M.D., Varvara Solodovnikova, M.D., Irina Liverko, Sc.D., Ronelle Moodliar, M.B., B.S., Matthew Dodd, M.Sc., Nosipho Ngubane, M.B., B.Ch., Mohammed Rassool, M.B., B.Ch., Timothy D. McHugh, Ph.D., Melvin Spigelman, M.D., David A.J. Moore, M.D., Koert Ritmeijer, Ph.D., Philipp du Cros, M.B., B.S., and Katherine Fielding, Ph.D., for the TB-PRACTECAL Study Collaborators*

ABSTRACT

BACKGROUND

In patients with rifampin-resistant tuberculosis, all-oral treatment regimens that are more effective, shorter, and have a more acceptable side-effect profile than current regimens are needed.

METHODS

We conducted an open-label, phase 2–3, multicenter, randomized, controlled, noninferiority trial to evaluate the efficacy and safety of three 24-week, all-oral regimens for the treatment of rifampin-resistant tuberculosis. Patients in Belarus, South Africa, and Uzbekistan who were 15 years of age or older and had rifampin-resistant pulmonary tuberculosis were enrolled. In stage 2 of the trial, a 24-week regimen of bedaquiline, pretomanid, linezolid, and moxifloxacin (BPaLM) was compared with a 9-to-20-month standard-care regimen. The primary outcome was an unfavorable status (a composite of death, treatment failure, treatment discontinuation, loss to follow-up, or recurrence of tuberculosis) at 72 weeks after randomization. The noninferiority margin was 12 percentage points.

RESULTS

Recruitment was terminated early. Of 301 patients in stage 2 of the trial, 145, 128, and 90 patients were evaluable in the intention-to-treat, modified intention-to-treat, and per-protocol populations, respectively. In the modified intention-to-treat analysis, 11% of the patients in the BPaLM group and 48% of those in the standard-care group had a primary-outcome event (risk difference, –37 percentage points; 96.6% confidence interval [CI], –53 to –22). In the per-protocol analysis, 4% of the patients in the BPaLM group and 12% of those in the standard-care group had a primary-outcome event (risk difference, –9 percentage points; 96.6% CI, –22 to 4). In the as-treated population, the incidence of adverse events of grade 3 or higher or serious adverse events was lower in the BPaLM group than in the standard-care group (19% vs. 59%).

CONCLUSIONS

In patients with rifampin-resistant pulmonary tuberculosis, a 24-week, all-oral regimen was noninferior to the accepted standard-care treatment, and it had a better safety profile. (Funded by Médecins sans Frontières; TB-PRACTECAL ClinicalTrials.gov number, NCT02589782.)

From the Public Health Department, Operational Center Amsterdam (OCA), Médecins sans Frontières, Amsterdam (B.-T.N., K.R.); the Public Health Department, OCA, Médecins sans Frontières (C.B., E.K., I.M.), the London School of Hygiene and Tropical Medicine (B.-T.N., M.D., D.A.J.M., K.F.), and University College London (T.D.M.) — all in London; the Republican Specialized Scientific and Practical Medical Center of Phthisiology and Pulmonology, Tashkent (N.P., I.L.), and the Republican Phthisiological Hospital No. 2, Nukus (Z.T.) — both in Uzbekistan; the Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus (V.S.); THINK TB and HIV Investigative Network, Durban (R.M.), and Wits Health Consortium, Johannesburg (N.N., M.R.) — both in South Africa; the Global Alliance for TB Drug Development, New York (M.S.); and the Burnet Institute, Melbourne, VIC, Australia (P.C.). Dr. Nyang'wa can be contacted at bern.nyangwa@london.msf.org or at Médecins sans Frontières, Operational Center Amsterdam, Plantage Middenlaan 14, 1018 DD Amsterdam, the Netherlands.

*A complete list of the TB-PRACTECAL study collaborators is provided in the Supplementary Appendix, available at NEJM.org.

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A Quick Take is available at NEJM.org

In 2019, approximately 465,000 patients had rifampin-resistant tuberculosis worldwide.[1] A total of 59% of the patients with rifampin-resistant tuberculosis who began receiving treatment in 2018 have had successful outcomes, and this incidence has not improved much in the past 5 years.[2]

The recommended duration of treatment for rifampin-resistant tuberculosis in programmatic care settings is 9 to 20 months[3] and involves up to 20 tablets per day. Cost,[4] adverse events,[5] and social disruption are prominent challenges. More effective, shorter treatments with a more acceptable side-effect profile are needed.[6] In a two-stage, phase 2–3 clinical trial (Pragmatic Clinical Trial for a more Effective, Concise and Less Toxic Regimen [TB-PRACTECAL]), we evaluated the safety and efficacy of 24-week, all-oral regimens for the treatment of rifampin-resistant tuberculosis.

In stage 1 of the trial, the primary objective was to identify regimens containing bedaquiline, pretomanid, and linezolid (BPaL) for evaluation in stage 2 on the basis of safety and efficacy at 8 weeks after randomization. The primary objective in stage 2 was to evaluate the safety and efficacy of a 24-week regimen containing BPaL plus moxifloxacin (BPaLM) for the treatment of rifampin-resistant tuberculosis. We report the outcomes of both stages of the trial as well as the results of additional analyses involving the groups that were not included in stage 2 of the trial.

METHODS

TRIAL DESIGN AND OVERSIGHT

We conducted an open-label, phase 2–3, multicenter, randomized, controlled noninferiority trial to compare the safety and efficacy of three investigational 24-week regimens with those of the accepted 9-to-20-month standard-care treatment for rifampin-resistant pulmonary tuberculosis. The trial was designed to seamlessly transition from a phase 2b trial to a phase 3 trial with one or two investigational groups. Further details are provided in Section S4 in the Supplementary Appendix and the protocol, both of which are available with the full text of this article at NEJM.org. The trial was approved by institutional ethics boards as well as local ethics committees and national regulatory authorities in the countries where the trial was conducted.

The trial was designed by the protocol development team (Section S1.1 in the Supplementary Appendix). The data were collected by the site investigators, and the statistical analysis was performed by the tenth and last authors and interpreted by all the authors. The first draft of the manuscript was written by the first four authors and the last author. All the authors participated in revision of the manuscript, approved the submitted versions, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

PATIENTS

Patients 15 years of age or older who had rifampin-resistant pulmonary tuberculosis were enrolled at seven sites in Belarus, South Africa, and Uzbekistan. The investigators were notified of new cases of laboratory-diagnosed rifampin-resistant tuberculosis from within the catchment areas.

The major inclusion criterion was *Mycobacterium tuberculosis* infection (as confirmed by a positive sputum smear) with resistance to rifampin. Patients were included irrespective of fluoroquinolone resistance, human immunodeficiency virus (HIV) status, or CD4 count. Patients were excluded if they were pregnant or if they had an alanine aminotransferase level or an aspartate aminotransferase level higher than 3 times the upper limit of the normal range, a corrected QT interval calculated with the use of Fridericia's formula (QTcF) greater than 450 msec, structural heart disease, or suspected resistance to bedaquiline, pretomanid, or linezolid. All the patients provided written informed consent.

TREATMENT

In stage 1 of the trial, enrolled patients were randomly assigned to the locally accepted standard-care treatment or to one of three investigational regimens. The standard-care regimen consisted of locally accepted treatment regimens. These regimens were closely aligned with the World Health Organization (WHO) guidelines for treatment of drug-resistant tuberculosis,[3] and the agents (some oral and some intravenous) were administered at least 6 days per week with food and under observation (see Section S5).

All the investigational agents were administered orally, with food and under observation, 7 days per week. The BPaL regimen consisted of the following: bedaquiline at a dose of 400 mg daily for 2 weeks, followed by 200 mg three

times per week for 22 weeks; pretomanid at a dose of 200 mg daily for 24 weeks; and linezolid at a dose of 600 mg daily for 16 weeks, followed by 300 mg daily for 8 weeks. The BPaLM regimen included BPaL plus moxifloxacin at a dose of 400 mg daily for 24 weeks, and the BPaLC regimen included BPaL plus clofazimine at a dose of 100 mg daily (or 50 mg if the patient weighed <30 kg) for 24 weeks. In stage 2 of the trial, patients were enrolled either into the standard-care group or into one of two investigational groups.

PROCEDURES

Patients were randomly assigned in a 1:1:1:1 ratio in stage 1 of the trial and in a 1:1 ratio in stage 2 of the trial (see the Supplementary Appendix). Randomization lists were prepared by the trial statisticians, and randomization was stratified according to trial site.

The trial schedule (see the protocol) included weekly visits for the first 2 weeks, monthly visits until week 24, and then visits every 2 months until week 108. Each visit included laboratory tests, three electrocardiographic assessments, and a physical examination that included a neurologic assessment. Assessments of visual acuity and color blindness and audiometric testing were also performed according to the schedule. The investigators assessed adverse events at each visit. Serious adverse events, adverse events of special interest, pregnancies, and overdoses that were identified were reported to the pharmacovigilance officer within 24 hours.

At inclusion and at scheduled time points, two sputum samples were obtained for smear microscopy and culture in liquid medium with the use of the Mycobacteria Growth Indicator Tube (MGIT) system (Becton Dickinson). Drug-susceptibility testing was performed in *M. tuberculosis* isolates that were obtained at baseline and in any samples that were culture positive at week 16 or later. Culture conversion was defined as at least one positive culture at baseline and at least two consecutive negative cultures obtained at least 2 weeks apart. Paired whole-genome sequencing was conducted in the event of treatment failure or recurrence of tuberculosis.

OUTCOMES

In stage 1 of the trial, the primary efficacy outcome was culture conversion in MGIT liquid medium at 8 weeks after randomization. The primary safety outcome was the incidence of death or discontinuation of treatment for any reason by week 8.

In stage 2 of the trial, the primary outcome was an unfavorable status (a composite of death, treatment failure, treatment discontinuation, loss to follow-up, or recurrence of tuberculosis) at 72 weeks after randomization. The secondary efficacy outcomes were culture conversion at 12 weeks, time to culture conversion, composite unfavorable outcomes at 24 weeks and 108 weeks after randomization, and recurrence of tuberculosis by week 48 after randomization (in the investigational groups only).

The safety outcomes in stage 2 of the trial were at least one serious adverse event or an adverse event of grade 3 or higher at 72 and 108 weeks after randomization and at the end of treatment and the incidence of prolongation of the QTcF interval at week 24. Deaths and adverse events of special interest were also reported.

ANALYSIS POPULATIONS

In the efficacy analyses, the intention-to-treat population included all patients who had undergone randomization. In the safety analyses, the as-treated population comprised all patients who had undergone randomization and received at least one dose of trial medication, and the patients were evaluated according to the regimen they received. The modified intention-to-treat population included patients in the intention-to-treat population who had received at least one dose of trial medication and excluded patients who did not have microbiologically proven rifampin-resistant tuberculosis. The per-protocol population included patients in the modified intention-to-treat population except those who did not complete a protocol-adherent course of treatment (>80% of doses within 120% of the prescribed duration) for any reason other than treatment failure or death and for those who discontinued treatment early because after they had received the first dose of trial treatment it was discovered that they had not met the inclusion or exclusion criteria.

Enrollment was terminated early for benefit, on March 18, 2021, in accordance with a recommendation from the data and safety monitoring board. We then performed an unplanned analysis, the results of which are presented here. In this analysis, the populations were restricted to include patients who could have had a prespecified

outcome event at a given time point (i.e., week 24, week 72, and week 108).

ADDITIONAL ANALYSES

After the stage 1 analysis, no analyses involving patients who were not included in stage 2 of the trial were planned. These patients were also followed to week 108, and these supportive data were viewed as important. All prespecified stage 2 analyses that involved the BPaLM group also were performed in the BPaLC and BPaL groups.

STATISTICAL ANALYSIS

The sample-size calculation is provided in the Supplementary Appendix. With the assumption that at 72 weeks after randomization 50% of the patients in the standard-care group and 45% of those in the investigational groups would have an unfavorable outcome event, we determined that a sample of 181 patients per group in trial stage 2 would provide the trial with approximately 85% power to detect a noninferiority margin of 12 percentage points. An alpha level of 1.7% (equivalent to a two-sided 96.6% confidence interval) was chosen to allow for both the adaptive nature of the design and the multiple comparisons of up to three groups. The estimated sample was increased to 201 patients per group to allow for patients who could not be evaluated. A noninferiority margin of 12 percentage points as the upper boundary of the confidence interval was determined to be a reasonable clinical and public health trade-off limit, given the benefits of a shorter treatment duration, decreased pill burden and regimen cost, and the all-oral nature of the investigational regimens. This noninferiority margin was congruent with that in recent trials involving patients with drug-resistant tuberculosis in which the noninferiority margin was 10 to 12 percentage points.[7,8]

The efficacy outcomes were analyzed in the intention-to-treat, modified intention-to-treat, and per-protocol populations, and the safety outcomes were analyzed in the as-treated population. Binary outcomes were summarized with absolute risk differences (with the use of a generalized linear model for a binomial outcome with an identity function) and risk ratios (with the use of a generalized linear model for a binomial outcome with a log-link function). Adjustment for randomization site was planned in all analyses. For the primary efficacy and safety outcomes, corresponding two-sided 96.6% confidence intervals were reported for effect estimates, and two-sided 95% confidence intervals were reported for secondary efficacy outcomes. The secondary outcomes were not adjusted for multiplicity. Prespecified subgroup analyses were conducted for the primary efficacy outcome. For binary safety outcomes, risk differences are reported with the use of the same approach as that described above. For the safety outcome of the QTcF value at 24 weeks, the difference in the mean value in each investigational group from the mean value in the standard-care group was assessed with adjustment for baseline QTcF values and with the use of linear regression.

Additional analyses of safety and efficacy were conducted in the BPaLC and BPaL groups with the use of the same approach but with two-sided 95% confidence intervals. Additional details are provided in the Supplementary Appendix.

Figure 1 (facing page). Trial Populations and Design.
Panel A shows the populations involved in the primary efficacy and safety analyses in stage 2 of the trial, including the patients who were excluded from the trial. Panel B shows the trial design. The trial was designed as a phase 2–3 clinical trial with a seamless transition from phase 2b to phase 3. Stage 1 included 240 patients with 60 patients in each group. A planned analysis involving the investigational groups only was then conducted to select groups for evaluation in stage 2. Evaluable patients included those who were enrolled in stage 1 and subsequently were included in the groups in stage 2. The first patient underwent randomization and the first visit occurred in January 2017, and stage 1 recruitment was completed in mid-2019. All three investigational groups met the eligibility criteria for progression to stage 2, but the trial steering committee elected to proceed with the BPaLM group only. Recruitment continued through the transition period across all four groups. This transition was delayed owing to the coronavirus disease 2019 pandemic. Recruitment was terminated for efficacy on March 18, 2021. Patients in all the groups underwent follow-up in accordance with the protocol for a minimum of 72 weeks after randomization. BPaL denotes bedaquiline, pretomanid, and linezolid; BPaLC bedaquiline, pretomanid, linezolid, and clofazimine; and BPaLM bedaquiline, pretomanid, linezolid, and moxifloxacin.

RESULTS

PATIENTS

The first patient underwent randomization in January 2017. A total of 552 patients were ran-

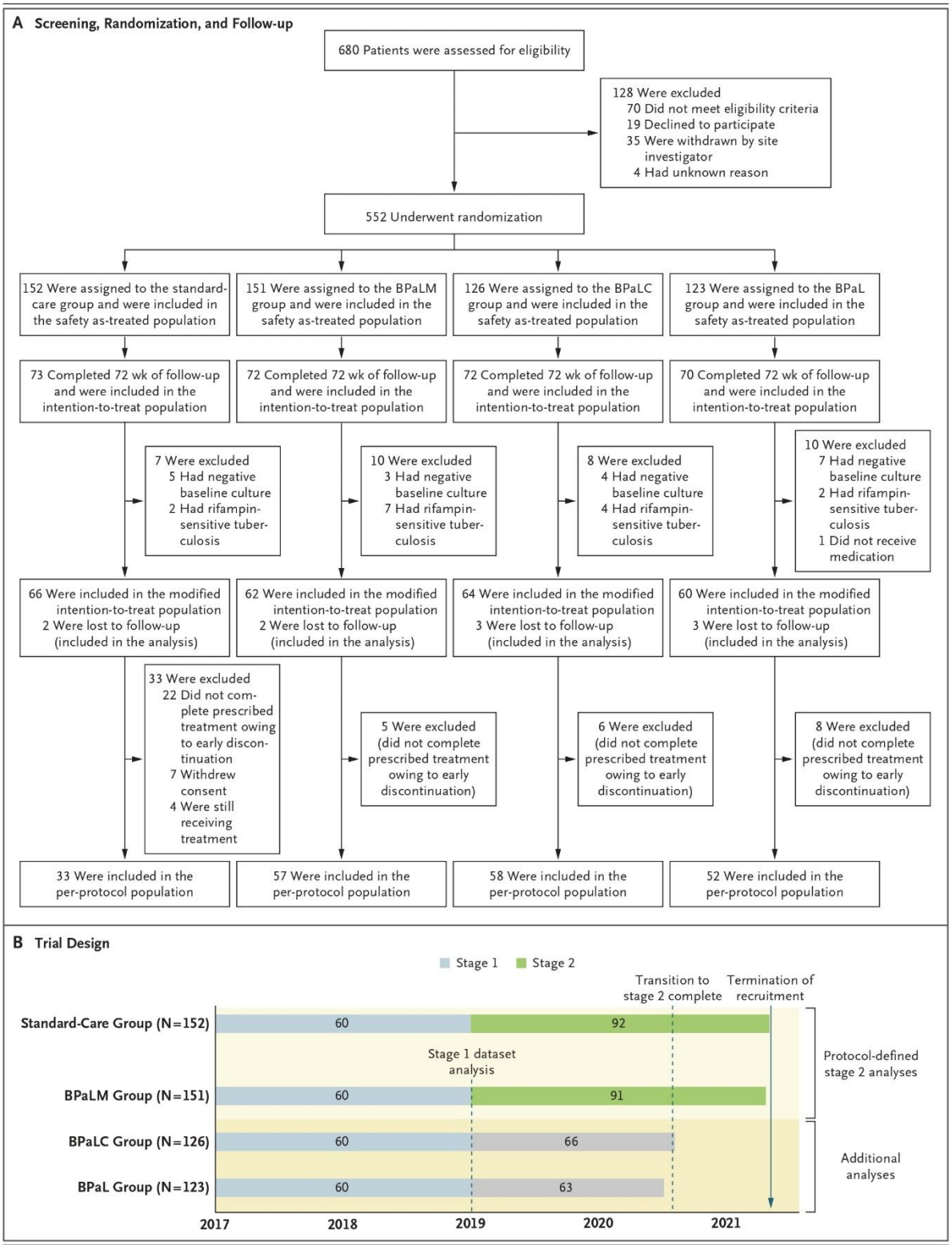

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Standard-Care Group	BPaLM Group	BPaLC Group	BPaL Group
Intention-to-treat population				
No. of patients	152	151	126	123
Geographic distribution — no. (%)				
Belarus	29 (19.1)	28 (18.5)	21 (16.7)	21 (17.1)
South Africa	54 (35.5)	56 (37.1)	48 (38.1)	47 (38.2)
Uzbekistan	69 (45.4)	67 (44.4)	57 (45.2)	55 (44.7)
Median age (range) — yr	37 (18–71)	35 (17–71)	32 (15–67)	35 (15–72)
Female sex — no. (%)	56 (36.8)	66 (43.7)	42 (33.3)	58 (47.2)
Median BMI (IQR)†	19.9 (17.3–22.8)	19.8 (17.7–22.7)	19.5 (17.7–22.2)	20.0 (18.1–22.4)
HIV-positive status — no. (%)	41 (27.0)	38 (25.2)	33 (26.2)	41 (33.3)
Median CD4 cell count (IQR) in HIV-infected patients — cells/mm^3‡	250 (132–460)	330 (209–547)	297 (114–481)	326 (153–550)
Smear positivity — no. (%)	98 (64.5)	91 (60.3)	84 (66.7)	77 (63)
Cavitation on chest radiography present — no. (%)	95 (62.5)	80 (53.0)	79 (62.7)	74 (60.2)
Fluoroquinolone-resistant tuberculosis — no./total no. (%)	32/131 (24.4)	32/134 (23.9)	22/118 (18.6)	25/104 (24.0)
QTcF interval — msec§	401±19	398±19	395±19	398±19
Median ALT level (IQR) — IU/liter¶	20 (15–28)	19 (14–28)	17 (14–26)	20 (14–31)
Modified intention-to-treat population with 72 wk of follow-up				
No. of patients	66	62	64	60
Geographic distribution — no. (%)				
Belarus	12 (18)	10 (16)	10 (16)	11 (18)
South Africa	18 (27)	16 (26)	19 (30)	16 (27)
Uzbekistan	36 (55)	36 (58)	35 (55)	33 (55)
Median age (range) — yr	36 (19–71)	34 (18–61)	29 (19–63)	34 (18–62)
Female sex — no. (%)	33 (50)	26 (42)	24 (38)	28 (47)
Median BMI (IQR)	19.2 (17.3–22.0)	19.8 (18.1–22.1)	18.8 (17.4–22.0)	20.5 (18.2–22.8)
HIV-positive status — no. (%)	15 (22.7)	14 (23)	14 (22)	14 (23)
Median CD4 cell count (IQR) — cells/mm^3‖	317 (154–383)	268 (182–364)	394 (112–511)	283 (153–424)
Smear positivity — no. (%)	50 (76)	40 (65)	43 (67)	45 (75)
Cavitation on chest radiography present — no. (%)	47 (71)	33 (53)	39 (61)	41 (68)
Fluoroquinolone-resistant tuberculosis — no./total no. (%)	18/65 (28)	17/60 (28)	16/62 (26)	19/56 (34)
QTcF interval — msec	398±18	396±18	393±20	398±18
Median ALT level (IQR) — IU/liter**	20 (15–27)	18 (14–27)	18 (15–27)	19 (14–27)

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding. The intention-to-treat population included all patients who had undergone randomization, and the modified intention-to-treat population included all patients in the intention-to-treat population who had received at least one dose of trial medication and excluded those who did not have microbiologically proven rifampin-resistant tuberculosis. ALT denotes alanine aminotransferase; BPaL bedaquiline, pretomanid, and linezolid; BPaLC bedaquiline, pretomanid, linezolid, and clofazimine; BPaLM bedaquiline, pretomanid, linezolid, and moxifloxacin; HIV human immunodeficiency virus; IQR interquartile range; and QTcF corrected QT interval, calculated with Fridericia's formula.

† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. Data on BMI were missing for one patient in the standard-care group.

‡ Data on CD4 cell count were missing for two patients each in the standard-care, BPaLM, and BPaL groups and for one patient in the BPaLC group.

§ Data on the QTcF interval were missing for one patient in the standard-care group.

¶ Data on the ALT level were missing for one patient each in the standard-care, BPaLM, and BPaLC groups.

‖ Data on the CD4 cell count were missing for one patient each in the standard-care, BPaLC, and BPaL groups.

** Data on the ALT level were missing for one patient in the BPaLM group.

Table 2. Primary Efficacy Analysis at 72 Weeks.

Variable	Intention-to-Treat Population		Modified Intention-to-Treat Population		Per-Protocol Population*	
	Standard-Care Group (N=73)	BPaLM Group (N=72)	Standard-Care Group (N=66)	BPaLM Group (N=62)	Standard-Care Group (N=33)	BPaLM Group (N=57)
Favorable outcome — no. (%)	34 (47)	55 (76)	34 (52)	55 (89)	29 (88)	55 (96)
Primary outcome: unfavorable status — no. (%)	39 (53)	17 (24)	32 (48)	7 (11)	4 (12)	2 (4)
Death — no. (%)	2 (3)	0	2 (3)	0	2 (6)	0
Early discontinuation — no. (%)	35 (48)	15 (21)	28 (42)	5 (8)	—	—
Adherence issues — no./total no. (%)	3/35 (9)	0	3/28 (11)	0	—	—
Adverse event — no./total no. (%)	17/35 (49)	5/15 (33)	17/28 (61)	5/5 (100)	—	—
Did not meet inclusion or exclusion criteria, detected after first dose — no./total no. (%)	7/35 (20)	10/15 (67)	0	0	—	—
Withdrew consent while still receiving treatment — no./total no. (%)	6/35 (17)	0	6/28 (21)	0	—	—
Other reason — no./total no. (%)†	2/35 (6)	0	2/28 (7)	0	—	—
Treatment failure — no.	0	0	0	0	0	0
Lost to follow-up at 72 wk — no. (%)	2 (3)	2 (3)	2 (3)	2 (3)	2 (6)	2 (4)
Recurrence — no.	0	0	0	0	0	0
Risk difference for the primary outcome — percentage points (96.6% CI)‡	—	−30 (−46 to −14)	—	−37 (−53 to −22)	—	−9 (−22 to 4)

* The per-protocol population included all patients in the modified intention-to-treat population with the exclusion of patients who did not complete a protocol-adherent course of treatment (>80% of doses within 120% of the prescribed duration), other than because of treatment failure or death, and patients who discontinued treatment early because they did not meet the inclusion or exclusion criteria.
† The "other outcome" category included one patient who could not be cared for at a trial site because of local regulations regarding infection control at the site and one patient who could not be cared for because the patient had acute behavioral challenges.
‡ The noninferiority margin was 12 percentage points on the difference scale.

domly assigned to one of the four groups; of these patients, 303 (54.9%) were included in the trial stage 2 groups (the standard-care group or the BPaLM group). On the date when enrollment was terminated, 145 patients (73 in the standard-care group and 72 in the BPaLM group) were included in the intention-to-treat population, 128 patients (66 in the standard-care group and 62 in the BPaLM group) were included in the modified intention-to-treat population, and 90 patients (33 in the standard-care group and 57 in the BPaLM group) were included in the per-protocol population. These patients could undergo 72 weeks of follow-up. In addition, of the patients who were originally assigned to one of four groups, 142 patients (72 in the BPaLC group and 70 in the BPaL group) in the intention-to-treat population, 124 patients (64 in the BPaLC group and 60 in the BPaL group) in the modified intention-to-treat population, and 110 patients (58 in the BPaLC group and 52 in the BPaL group) in the per-protocol population could undergo 72 weeks follow-up as well as additional evaluations (Fig. 1A and 1B).

The baseline demographic characteristics of the patients were generally balanced among the trial groups in the intention-to-treat, modified intention-to-treat, and per-protocol populations that underwent follow-up for 72 weeks. In the modified intention-to-treat analysis, the standard-care group had a higher proportion of female patients and patients with smear-positive and cavitary disease than the investigational groups (Table 1). Most patients in the standard-care group received at least two WHO group A drugs[3] as part of their regimen (Table S7); these drugs were fluoroquinolones (in 95%), linezolid (in 77%), and bedaquiline (in 76%).

EFFICACY OUTCOMES

In stage 1 of the trial, the percentages of patients with culture conversion in liquid medium at 8 weeks after randomization were 77%, 67%, and 46% in the BPaLM, BPaLC, and BPaL groups, respectively (Table S8 in the Supplementary Appendix); 8%, 6%, and 10% of the patients, respectively, discontinued treatment or died. The BPaLM regimen was selected for analysis in stage 2 of the trial.

In stage 2, by 72 weeks of follow-up in the intention-to-treat population, 39 of 73 patients in the standard-care group (53%) and 17 of 72 of

Table 3. Outcomes at 72 Weeks in the Standard-Care, BPaLC, and BPaL Groups.*

Variable	Intention-to-Treat Population			Modified Intention-to-Treat Population			Per-Protocol Population		
	Standard-Care Group (N=73)	BPaLC Group (N=72)	BPaL Group (N=70)	Standard-Care Group(N=66)	BPaLC Group (N=64)	BPaL Group (N=60)	Standard-Care Group(N=33)	BPaLC Group (N=58)	BPaL Group (N=52)
Favorable outcome — no. (%)	34 (47)	52 (72)	46 (66)	34 (52)	52 (81)	46 (77)	29 (88)	52 (90)	46 (88)
Primary outcome: unfavorable status — no. (%)	39 (53)	20 (28)	24 (34)	32 (48)	12 (19)	14 (23)	4 (12)	6 (10)	6 (12)
Death — no. (%)	2 (3)	1 (1)	0	2 (3)	1 (2)	0	2 (6)	1 (2)	0
Early discontinuation — no. (%)	35 (48)	14 (19)	18 (26)	28 (42)	6 (9)	8 (13)	—	—	—
Adherence issues — no./total no. (%)	3/35 (9)	2/14 (14)	2/18 (11)	3/28 (11)	2/6 (33)	2/8 (25)	—	—	—
Adverse event — no./total no. (%)	17/35 (49)	4/14 (29)	5/18 (28)	17/28 (25)	4/6 (67)	5/8 (62)	—	—	—
Did not meet inclusion or exclusion criteria, detected after first dose — no./total no. (%)	7/35 (20)	8/14 (57)	10/18 (6)	0	0	1/8 (12)	—	—	—
Did not receive at least one dose of trial medication — no./total no. (%)	0	0	1/18 (6)	—	—	—	—	—	—
Withdrew consent while still receiving treatment — no./total no. (%)	6/35 (17)	0	0	6/28 (21)	0	0	—	—	—
Other reason — no./total no. (%)†	2/35 (6)	0	0	2/28 (7)	0	0	—	—	—
Treatment failure — no. (%)	0	1 (1)	0	0	1 (2)	0	0	1 (2)	0
Lost to follow-up at 72 wk — no. (%)	2 (3)	3 (4)	3 (4)	2 (3)	3 (5)	3 (5)	2 (6)	3 (5)	3 (6)
Recurrence — no. (%)	0	1 (1)	3 (4)	0	1 (2)	3 (5)	0	1 (2)	3 (6)
Risk difference for the primary outcome — percentage points (95% CI)	—	−26 (−41 to −10)	−19 (−36 to −2)	—	−30 (−45 to −14)	−25 (−41 to −9)	—	−2 (−15 to 12)	−1 (−15 to 14)

* Confidence intervals for the BPaLC group and BPaL group as compared with the standard-care group are two-sided and were not adjusted for multiplicity and should not be used to infer relative treatment effects.

† The "other outcome" category included one patient who could not be cared for at a trial site because of local regulations regarding infection control at the site and one patient could not be cared for because the patient had acute behavioral challenges.

patients in the BPaLM group (24%) had an unfavorable status (the primary composite outcome). In the modified intention-to-treat population, 32 of 66 patients in the standard-care group (48%) and 7 of 62 patients in the BPaLM group (11%) had an unfavorable status. The unadjusted risk difference was −37 percentage points (96.6% confidence interval [CI], −53 to −22), and the BPaLM regimen was both noninferior and superior to the standard regimen. In the per-protocol population, 4 of 33 patients in the standard-care group (12%) and 2 of 57 patients in the BPaLM group (4%) had an unfavorable status. No recurrences of tuberculosis or treatment failures were detected in either group (Table 2).

There was no evidence that treatment effects varied according to age, sex, HIV infection, sputum smear status, the presence of cavities on chest radiographs, fluoroquinolone resistance, or country of recruitment in the subgroup analyses. More details are provided in Table S22.

In stage 2, with regard to the secondary efficacy outcomes, the risk of a composite unfavorable outcome event at 24 and 108 weeks was broadly consistent with that with the primary outcome. In the modified intention-to-treat population, 78 of 99 patients in the standard-care group (79%) and 85 of 96 patients in the BPaLM group (88%) had culture conversion at 12 weeks; these results were similar in the per-protocol population. In a time-to-event analysis, the hazard ratio for culture conversion was 1.59 (95% CI, 1.18 to 2.14) in the modified intention-to treat population and 1.67 (95% CI, 1.14 to 2.45) in the per-protocol population (Table S13). At week 48, there were no recurrences of tuberculosis in the BPaLM group.

In additional efficacy analyses, by 72 weeks of follow-up in the modified intention-to-treat population, 12 of 64 patients in the BPaLC group (19%) and 14 of 60 patients in the BPaL group (23%) had an unfavorable composite outcome event. The unadjusted risk difference as compared with standard care was −30 percentage points (95% CI, −45 to −14) in the BPaLC group and −25 percentage points (95% CI, −41 to −9) in the BPaL group. In the per-protocol population, 6 of 58 patients in the BPaLC group (10%) and 6 of 52 patients in the BPaL group (12%) had an unfavorable composite outcome event. The unadjusted risk difference as compared with the standard of care was −2 percentage points (95% CI, −15 to 12) in the BPaLC group and −1 percentage point (95% CI, −15 to 14) in the BPaL group. In the per-protocol population, one treatment failure and one tuberculosis recurrence were observed in the BPaLC group; in the BPaL group, three tuberculosis recurrences were observed (Table 3).

SAFETY OUTCOMES

By 72 weeks of follow-up, 43 of 73 patients in the standard-care group (59%) had a total of 69 events (at least one serious adverse event or an adverse event of grade ≥3), and 14 of 72 patients in the BPaLM group (19%) had a total of 16 events (risk difference, −40 percentage points; 96.6% CI, −55 to −24). At least one serious adverse event or an adverse event of grade 3 or higher occurred in 23 of 72 patients (32%; 32 events) in the BPaLC group and 15 of 69 patients (22%; 24 events) in the BPaL group (Table 4).

By 72 weeks, the most frequently observed serious or grade 3 or higher adverse events were hepatic disorders. These affected 8 of 73 patients in the standard-care group (11%), 3 of 72 patients in the BPaLM group (4%), 3 of 72 patients in the BPaLC group (4%), and 2 of 69 patients in the BPaL group (3%). None of the patients in any of the groups met the Hy's law criteria for drug-induced liver injury (Fig. S4).

QTcF prolongation, the second most frequent serious or grade 3 or higher adverse event, affected 14 patients: 10 of 73 patients in the standard-care group (14%), 1 of 72 patients in the BPaLM group (1%), 3 of 72 patients in the BPaLC group (4%), and none of the patients in the BPaL group. QTcF prolongation for more than 500 msec led to early discontinuation of treatment in 6 patients in the standard-care group and in 1 patient in any of the investigational groups (the BPaLC group). At 24 weeks after randomization, the mean difference in a QTcF from the standard-care group, with adjustment for baseline QT, was −18.1, −5.4, and −20.0 msec in the BPaLM group, BPaLC group, and BPaL group, respectively.

Peripheral neuropathy (any grade) was seen in 28 of 150 patients in the standard-care group (19%; a total of 33 events), in 14 of 151 patients in the BPaLM group (9%; a total of 15 events), in 10 of 126 patients in the BPaLC group (8%; a total of 10 events), and in 16 of 122 patients in the BPaL group (13%; a total of 19 events). A

Table 4. Safety Outcomes (As-Treated Population).*

Variable	Standard-Care Group	BPaLM Group	BPaLC Group	BPaL Group
QTcF interval at 24 wk				
No. of patients with data†	71	98	92	92
QTcF interval at 24 wk — msec	441.8±18.0	423.5±18.5	435.7±17.6	423.1±18.5
Mean difference (CI) — msec‡§	—	−18.1 (−23.4 to −12.8)	−5.4 (−10.3 to −0.6)	−20.0 (−25.1 to −14.9)
Serious adverse event or grade ≥3 adverse event within 108 wk after randomization				
Patients with at ≥1 event — no./total no. (%)	26/43 (60)	10/40 (25)	18/43 (42)	11/43 (26)
No. of events	48	11	22	21
Risk difference — percentage points CI)§	—	−36 (−57 to −14)	−19 (−39 to 2)	−35 (−54 to −15)
Serious adverse event or grade ≥3 adverse events during treatment and up to 30 days after treatment end date				to
Patients with ≥1 event — no./total no. (%)	25/43 (58)	7/40 (18)	11/43 (26)	10/43 (23)
No. of events	46	7	14	12
Risk difference — percentage points (CI)§	—	−41 (−61 to −20)	−33 (−52 to −13)	−35 (−54 to −16)
Serious adverse event or grade ≥3 adverse events within 72 wk after randomization				
Patients with at ≥1 event — no./total no. (%)	43/73 (59)	14/72 (19)	23/72 (32)	15/69 (22)
No. of events	69	16	32	24
Risk difference — percentage points (CI)§	—	−40 (−55 to −24)	−27 (−43 to −11)	−37 (−52 to −22)
Hepatic disorder, grouped				
No. of events	10	3	5	2
Patients with events — no./total no. (%)	8/73 (11)	3/72 (4)	3/72 (4)	2/69 (3)
QTcF prolongation¶‖				
No. of events	12	1	3	0
Patients with events — no./total no. (%)	10/73 (14)	1/72 (1)	3/72 (4)	0
Creatinine renal clearance decreased				
No. of events	7	1	0	2
Patients with events — no./total no. (%)	5/73 (7)	1/72 (1)	0	2/69 (3)
Anemia				
No. of events	6	2	0	1
Patients with events — no./total no. (%)	6/73 (8)	2/72 (3)	0	1/69 (1)
Neutropenia				
No. of events	2	3	0	0
Patients with events — no./total no. (%)	2/73 (3)	3/72 (4)	0	0
Lipase level increased or pancreatitis				
No. of events	1	2	2	2
Patients with events — no./total no. (%)	1/73 (1)	2/72 (3)	2/72 (3)	2/69 (3)
Acute kidney injury				
No. of events	1	1	0	1
Patients with events — no./total no. (%)	1/73 (1)	1/72 (1)	0	1/69 (1)

Table 4. (Continued.)

Variable	Standard-Care Group	BPaLM Group	BPaLC Group	BPaL Group
Hemoptysis‖				
No. of events	2	0	1	0
Patients with events — no./total no. (%)	1/73 (1)	0	1/72 (1)	0
Vomiting				
No. of events	2	0	0	0
Patients with events — no./total no. (%)	2/73 (3)	0	0	0
Lymphocyte count decreased				
No. of events	0	1	1	1
Patients with events — no./total no. (%)	0	1/72 (1)	1/72 (1)	1/69 (1)
Pneumonia				
No. of events	1	0	2	1
Patients with events — no./total no. (%)	1/73 (1)	0	2/72 (3)	1/69 (1)
Other‖				
No. of events	25	2	18	14
Patients with events — no./total no. (%)	23/73 (32)	2/72 (3)	18/72 (25)	12/69 (17)

* Plus–minus values are means ±SD. The as-treated population included all patients who underwent randomization and received at least one dose of trial medication.
† This category excludes patients who were not participating in the trial at week 24, even if they discontinued owing to QTcF prolongation.
‡ The mean difference was adjusted for the baseline QTcF interval.
§ Confidence intervals for the BPaLM group group as compared with the standard-care group are two-sided 96.6% confidence intervals. Confidence intervals for the BPaLC group and BPaL group as compared with the standard-care group are two-sided 95% confidence intervals and are not adjusted for multiplicity.
¶ QTcF prolongation includes prolonged QT on electrocardiography and syncope.
‖ One patient had two events.

single event of grade 3 peripheral neuropathy occurred in a patient in the standard-care group 75 days after randomization. No episodes of optic neuropathy were observed.

Ten of the 549 patients (2%) in the as-treated population died; 7 of these patients were in the standard-care group. Four patients died during the treatment period, 3 died during follow-up, and 3 died after early withdrawal from the trial. Four of the deaths (all in the standard-care group) were considered by the investigators to be treatment-related. None of the deaths were attributed by the investigators to tuberculosis (Table S20).

DISCUSSION

In the modified-intention-to-treat population in this phase 2–3 trial, BPaLM was both noninferior and superior to the accepted standard care with respect to the primary composite outcome; 89% and 52% of the patients, respectively, had a favorable outcome. The percentages of patients with favorable outcomes in the BPaLC group (81%) and the BPaL group (77%) were also higher than the percentage in the standard-care group. The difference was principally driven by early discontinuation of treatment owing to adverse events in the standard-care group. The difference between the standard-care and investigational groups was less pronounced in the per-protocol analysis in which early discontinuations were excluded. These findings suggest that the standard-care treatment was similarly efficacious when patients could receive it without adverse effects.

The safety outcomes also favored BPaLM, with lower percentages of patients with adverse events of grade 3 or higher or serious adverse

events for all outcomes (at week 72, at week 108, and during treatment). In additional safety analyses, the BPaLC and BPaL regimens were also safer than the standard care. The QTcF interval at week 24 was lower in the BPaLM group than in the standard-care group and more closely resembled the QTcF in the BPaL group. The QTcF in the BPaLC group was similar to that in the standard-care group. This finding corroborates evidence suggesting that clofazimine is a primary driver of QTcF prolongation in bedaquiline-containing regimens.

These findings are generally consistent with those from other trials of shorter bedaquiline, pretomanid, and linezolid regimens.[9,10] In those trials, 84 to 93% of the patients had a successful outcome, percentages that were similar to those in trials involving patients with drug-sensitive tuberculosis.[11] In our trial, BPaL did not appear to perform as well as the regimen in the Nix-TB study,[9] with fewer successful outcomes and slower culture conversion. The trial design may explain this difference (Table S27).

These results are also consistent with data from trials of other shorter regimens. In the STREAM (Standard Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB) trial, 78.8% of patients in the short-regimen group had a successful outcome.[7] A meta-analysis of the current 9-to-11-month all-oral regimen recommended by the WHO showed a successful outcome in 73% of patients.[12] A retrospective study of a shorter regimen including linezolid showed a successful outcome in 75.2% of patients.[13] Although the percentage of patients with unfavorable outcomes in the standard-care group in our trial is consistent with those reported worldwide,[1,2] it is lower than what has been reported in recent clinical trials involving patients with drug-resistant tuberculosis.[7] Enhanced monitoring and stringent discontinuation criteria in our trial probably explain this difference. The criteria for discontinuation were applied to all groups equally.

Our trial has several strengths. This randomized, controlled, regulatory-level trial enrolled patients who were broadly representative of patients in the epidemic of rifampin-resistant tuberculosis, with the inclusion of patients with fluoroquinolone-resistant tuberculosis and HIV coinfection (Table S2). The trial was patient-centered, with assistance in adherence to treatment adapted to the patients' circumstances. The safety of patients was paramount, with frequent visits to ensure that adverse events were identified and managed promptly. These visits were complemented by centralized safety oversight. TB-PRACTECAL substudies are also under way to provide explanatory data, specifically regarding the costs of new regimens for patients and providers, as well as their cost-effectiveness and effect on patients' poverty levels,[14] patient-reported outcomes,[15] and pharmacokinetics and pharmacodynamics.[16]

The TB-PRACTECAL trial was terminated for efficacy after recruitment of 75% of the planned sample. Trials that are terminated early for benefit have been suggested to overestimate treatment effects,[17] although it has been argued that this overestimation is limited.[18] Recruitment into our trial was terminated on the recommendation of the data and safety monitoring board after the prespecified stopping rule was triggered.[19] A study of follow-up data for at least 72 weeks after randomization in all patients who underwent randomization is under way.

The limitations of our trial include the open-label design. Poorer performance of the standard-care treatment was driven by early discontinuations in the modified intention-to-treat population, but the criteria for discontinuation owing to poor adherence to treatment or adverse events were prespecified (see the protocol). Although 17 of the 28 discontinuations in the standard-care group in the modified intention-to-treat population were due to adverse events, the remainder could have been subject to performance bias. Seven patients withdrew consent in the standard-care group while receiving treatment. Our inability to measure minimum inhibitory concentrations in all patients for this report limited the subgroup analyses. We were unable to perform whole-genome sequencing at the trial site where the recurrences of tuberculosis occurred, so we cannot rule out the possibility that these recurrences were caused by reinfection. The standard-care regimens were updated throughout the trial, in line with international recommendations. However, these changes meant that the standard care differed over time and according to trial site. Current standard-care regimens include less toxic drugs than those used earlier in the trial.[5] Of note, most patients in the standard-care group received at least two WHO

group A drugs[3] as part of their regimen, an approach consistent with current guidelines. As planned, the data and safety monitoring board reviewed summary data every 3 to 6 months to ensure adequate oversight. In November 2020, the data and safety monitoring board requested the treatment effect and confidence interval for the composite outcome; no adjustment in the alpha level was made for this analysis.

This multicountry, randomized, controlled trial of 24-week, all-oral regimens containing bedaquiline, pretomanid, and linezolid for the treatment of rifampin-resistant tuberculosis showed that treatment with BPaLM was more effective and had a better safety profile than standard care. BPaLC and BPaL were also highly efficacious.

Supported by Médecins sans Frontières. The TB Alliance donated the first batch of pretomanid before it was commercially available.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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2.4. Paper 3 – TB-PRACTECAL clinical trial final results

Nyang'wa BT, Berry C, Kazounis E, Motta I, Parpieva N, Tigay Z, Moodliar R, Dodd M, Solodovnikova V, Liverko I, Rajaram S, Rassool M, McHugh T, Spigelman M, Moore DA, Ritmeijer K, du Cros P, Fielding K; TB-PRACTECAL team. Short oral regimens for pulmonary rifampicin-resistant tuberculosis (TB-PRACTECAL): an open-label, randomised, controlled, phase 2B-3, multi-arm, multicentre, non-inferiority trial. Lancet Respir Med. 2023 Nov 15:S2213-2600(23)00389-2. doi: 10.1016/S2213-2600(23)00389-2.

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SECTION A – Student Details

Student ID Number	1700547	**Title**	Dr
First Name(s)	Bern-Thomas		
Surname/Family Name	Nyang'wa		
Thesis Title	Short, effective and safe all-oral treatment for rifampicin resistant tuberculosis: TB-PRACTECAL trial and its drugs pharmacokinetics		
Primary Supervisor	Prof. David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Lancet Respiratory Medicine		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I have been the Chief Investigator and Project Manager of the TB-PRACTECAL trial. In this role, I led the conceptualisation of the TB-PRACTECAL randomised controlled trial, development of the trial protocol including chairing the protocol writing committee, oversaw the implementation of the protocol, data collection, data analysis, interpretation and results communication. I steered the research project and was the principal decision maker on the study implementation choices (site selection, clinical management guidance, data collection tools, quality assurance approaches etc) and analysis plan. I prepared together with some authors the first draft manuscript, communicated with the journal throughout revisions and approved the final manuscript

SECTION E

Student Signature	[redacted]
Date	Jun 17, 2024

Supervisor Signature	[redacted]
Date	Jun 17, 2024

Short oral regimens for pulmonary rifampicin-resistant tuberculosis (TB-PRACTECAL): an open-label, randomised, controlled, phase 2B-3, multi-arm, multicentre, non-inferiority trial

*Bern-Thomas Nyang'wa, Catherine Berry, Emil Kazounis, Ilaria Motta, Nargiza Parpieva, Zinaida Tigay, Ronelle Moodliar, Matthew Dodd, Varvara Solodovnikova, Irina Liverko, Shakira Rajaram, Mohammed Rassool, Timothy McHugh, Melvin Spigelman, David A Moore, Koert Ritmeijer, Philipp du Cros, Katherine Fielding, on behalf of the TB-PRACTECAL team**

Summary

Background Around 500 000 people worldwide develop rifampicin-resistant tuberculosis each year. The proportion of successful treatment outcomes remains low and new treatments are needed. Following an interim analysis, we report the final safety and efficacy outcomes of the TB-PRACTECAL trial, evaluating the safety and efficacy of oral regimens for the treatment of rifampicin-resistant tuberculosis.

Methods This open-label, randomised, controlled, multi-arm, multicentre, non-inferiority trial was conducted at seven hospital and community sites in Uzbekistan, Belarus, and South Africa, and enrolled participants aged 15 years and older with pulmonary rifampicin-resistant tuberculosis. Participants were randomly assigned, in a 1:1:1:1 ratio using variable block randomisation and stratified by trial site, to receive 36–80 week standard care; 24-week oral bedaquiline, pretomanid, and linezolid (BPaL); BPaL plus clofazimine (BPaLC); or BPaL plus moxifloxacin (BPaLM) in stage one of the trial, and in a 1:1 ratio to receive standard care or BPaLM in stage two of the trial, the results of which are described here. Laboratory staff and trial sponsors were masked to group assignment and outcomes were assessed by unmasked investigators. The primary outcome was the percentage of participants with a composite unfavourable outcome (treatment failure, death, treatment discontinuation, disease recurrence, or loss to follow-up) at 72 weeks after randomisation in the modified intention-to-treat population (all participants with rifampicin-resistant disease who received at least one dose of study medication) and the per-protocol population (a subset of the modified intention-to-treat population excluding participants who did not complete a protocol-adherent course of treatment (other than because of treatment failure or death) and those who discontinued treatment early because they violated at least one of the inclusion or exclusion criteria). Safety was measured in the safety population. The non-inferiority margin was 12%. This trial is registered with ClinicalTrials.gov, NCT02589782, and is complete.

Findings Between Jan 16, 2017, and March 18, 2021, 680 patients were screened for eligibility, of whom 552 were enrolled and randomly assigned (152 to the standard care group, 151 to the BPaLM group, 126 to the BPaLC group, and 123 to the BPaL group). The standard care and BPaLM groups proceeded to stage two and are reported here, post-hoc analyses of the BPaLC and BPaL groups are also reported. 151 participants in the BPaLM group and 151 in the standard care group were included in the safety population, with 138 in the BPaLM group and 137 in the standard care group in the modified intention-to-treat population. In the modified intention-to-treat population, unfavourable outcomes were reported in 16 (12%) of 137 participants for whom outcome was assessable in the BPaLM group and 56 (41%) of 137 participants in the standard care group (risk difference −29·2 percentage points [96·6% CI −39·8 to −18·6]; non-inferiority and superiority p<0·0001). 34 (23%) of 151 participants receiving BPaLM had adverse events of grade 3 or higher or serious adverse events, compared with 72 (48%) of 151 participants receiving standard care (risk difference −25·2 percentage points [96·6% CI −36·4 to −13·9]). Five deaths were reported in the standard care group by week 72, of which one (COVID-19 pneumonia) was unrelated to treatment and four (acute pancreatitis, suicide, sudden death, and sudden cardiac death) were judged to be treatment-related.

Interpretation The 24-week, all-oral BPaLM regimen is safe and efficacious for the treatment of pulmonary rifampicin-resistant tuberculosis, and was added to the WHO guidance for treatment of this condition in 2022. These findings will be key to BPaLM becoming the preferred regimen for adolescents and adults with pulmonary rifampicin-resistant tuberculosis.

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed for articles published in English between Jan 1, 2006, and Jan 16, 2017, using the search terms "bedaquiline" AND "pretomanid" AND "linezolid". We found nine articles, none of which reported treatment outcomes of regimens comprising bedaquiline, pretomanid, and linezolid. One was excluded as no abstract was available; three reported on preclinical studies, none of which reported on the bedaquiline, pretomanid, and linezolid (BPaL) regimen. Five were reviews of new anti-tuberculosis drugs and the design of planned and ongoing tuberculosis studies, only one of which referred to the design of a BPaL regimen study (NiX-TB); the others described studies of the pretomanid, moxifloxacin, and pyrazinamide regimen. To our knowledge, TB-PRACTECAL is the first randomised controlled trial of 24-week regimens containing bedaquiline, pretomanid, and linezolid (BPaL), BPaL plus clofazimine (BPaLC), and BPaL plus moxifloxacin (BPaLM) for the treatment of rifampicin-resistant tuberculosis. At the time of protocol finalisation in June, 2016, only varying results regarding the clinical safety and efficacy of the component drugs had been published. Personal correspondence (Spigelman M, Global Alliance for TB Drug Development, New York, NY, USA) was available on the preliminary outcomes of the NiX-TB study, which was later published in 2020, showing that treatment with BPaL for 6–9 months led to favourable outcomes in 90% of participants with highly drug-resistant forms of tuberculosis. In 2022, the interim analysis of the TB-PRACTECAL study was published, showing fewer unfavourable outcomes in the BPaLM group than in the standard care group (risk difference −37·2 percentage points [96·6% CI −52·8 to −21·6]). The analysable modified intention-to-treat populations in the interim analysis comprised 66 patients in the standard of care group and 62 patients in the BPaLM group.

Added value of this study

This final analysis of the TB-PRACTECAL trial substantiates, with improved precision, the non-inferiority of the BPaLM regimen when compared with the standard of care. The majority of participants (95 [69%] of 137) included in the control group of this final analysis received an improved standard treatment, in line with 2019 WHO recommendations, and the modified intention-to-treat populations were larger than those in the interim analysis, comprising 137 participants in the standard care group and 138 participants in the BPaLM group.

Implications of all the available evidence

These data add strength to the WHO recommendation to include BPaLM as a preferred regimen for treatment of adolescents and adults with pulmonary rifampicin-resistant tuberculosis. The duration of treatment is now in line with that of most regimens for the treatment of drug-sensitive tuberculosis.

Introduction

Each year, around 500 000 people worldwide develop rifampicin-resistant tuberculosis, defined as tuberculosis disease that is resistant to at least rifampicin. Until 2020, treatment was 9–20 months in duration, had considerable toxicity, and was of inadequate effectiveness. In 2022, successful outcomes were reported for only 60% of patients who started treatment for rifampicin-resistant tuberculosis.[1]

The TB-PRACTECAL trial was designed to examine if combinations of new and repurposed antitubercular drugs could provide effective 24-week treatment regimens for rifampicin-resistant tuberculosis that were at least non-inferior to standard care.

In a multi-arm, multistage trial, three candidate regimens were considered, containing bedaquiline, linezolid, and pretomanid (BPaL) with and without the addition of either moxifloxacin (BPaLM) or clofazimine (BPaLC).[2] After a planned first-stage analysis, the BPaLM group was the most promising based on phase 2B efficacy and safety findings.[3]

In 2022, after early termination of the trial for efficacy, WHO convened a guideline development group to consider the interim data. The interim analysis of data collected up to early termination showed that BPaLM was superior to standard care.[3,4] On this basis, the guidance development group concluded that a BPaLM regimen for 6 months should be the preferred regimen for the treatment of rifampicin-resistant tuberculosis without additional resistance to fluoroquinolones, and the BPaL regimen[5] was recommended for rifampicin-resistant tuberculosis with additional resistance to fluoroquinolones.

After the termination of recruitment on March 18, 2021, participants were followed up for at least 72 weeks from randomisation. Here we present the final analysis of the TB-PRACTECAL trial, evaluating the safety and efficacy of the 24-week BPaLM regimen compared with standard care.

Methods

Study design

We conducted an open-label, randomised, controlled, multi-arm, multicentre, non-inferiority trial at seven hospital and community sites in Uzbekistan, Belarus, and South Africa. The trial was designed to transition from a phase 2B (stage one) to a phase 3 (stage two) trial with up to two investigational groups. Recruitment to all four groups continued throughout the transition period provided that the data safety monitoring board had no concerns. The scientific advisory committee was provided masked efficacy and safety data at the end of stage one and, on this basis, recommended which investigational groups should progress to stage two for phase 3

evaluation (appendix p 5). Details of the protocol and trial conduct have been previously published.[2]

Ethics approval was obtained from two central institutional ethics boards (London School of Hygiene & Tropical Medicine Research Ethics Committee and Médecins Sans Frontières Ethics Review Board) as well as local ethics committees and national regulatory authorities in Belarus, South Africa, and Uzbekistan.

Participants

Investigators were notified by laboratory staff of new patients with microbiologically diagnosed rifampicin-resistant tuberculosis from within the catchment areas of the trial sites. Patients aged 15 years or older who had pulmonary *Mycobacterium tuberculosis* disease, with rifampicin resistance confirmed by molecular or culture-based drug susceptibility testing, were offered enrolment. Participants were included irrespective of fluoroquinolone resistance status, HIV status, or CD4 count.

Patients were excluded if they were pregnant or if they had an alanine aminotransferase concentration or an aspartate aminotransferase concentration higher than three times the upper limit of the normal range; a Fridericia-corrected QT (QTcF) interval longer than 450 ms; structural heart disease; or a known or high risk of resistance to bedaquiline, pretomanid, or linezolid. Sex was self-reported with binary options. Full inclusion and exclusion criteria have previously been described.[2] All participants provided written informed consent.

Randomisation and masking

Randomisation lists were computer-generated and prepared by the trial statisticians. Using variable block randomisation, participants were randomly assigned in a 1:1:1:1 ratio to receive standard care, BPaL, BPaLC, or BPaLC in stage one of the trial, and in a 1:1 ratio to receive standard care or BPaLM in stage two of the trial. Participants were stratified by trial site. For allocation concealment, sites used sequentially numbered opaque envelopes at the start of the trial, but subsequently transitioned to computer assignment. After enrolment by investigators, randomisation was conducted by trial site pharmacists who notified investigators of the treatment allocation. Site pharmacists had no other direct role in participant care.

The trial was open-label. Trial site laboratory staff and central sponsor staff were masked to group assignment.

After the early termination of recruitment on March 18, 2021, all participants were notified that the trial had been terminated for benefit. Participants in the standard care group with at least 6 months remaining before completion of their intended regimen were given the option to cross over to the BPaLM group. Investigators and participants were given the discretion to individualise this decision in accordance with the wishes and best interests of the participant. Participants in the BPaLC and BPaL groups continued their allocated treatments.

Procedures

All participants allocated to the investigational groups were prescribed BPaL as the backbone of the regimens, comprising linezolid 600 mg daily for 16 weeks and 300 mg daily for 8 weeks (the lower dose was started earlier if the higher dose was not sufficiently well tolerated), pretomanid 200 mg daily for 24 weeks, and bedaquiline 400 mg daily for 2 weeks followed by 200 mg three times per week for 22 weeks. Participants in the BPaLM group were given BPaL plus moxifloxacin 400 mg daily and those in the BPaLC group were given BPaL plus clofazimine 100 mg daily (or 50 mg if weight <33 kg). Treatment duration was 24 weeks and all drugs were administered orally. Participants allocated to the standard care group were treated according to the locally accepted standard of care, which was continuously updated in line with WHO guidance. At the start of the trial, standard care regimens included both shorter, standardised 9–11-month (36–44-week) regimens as well as longer, individualised 18–20-month (72–80 week) regimens. From 2017 to 2019, these regimens generally included a second-line injectable agent and criteria for including bedaquiline were stringent. From 2019, participants received all-oral versions of these regimens and most regimens included bedaquiline. In South Africa, a 9–11-month regimen was used from 2018 and was subsequently approved by WHO in 2022.[3] Treatment was prescribed by investigators in line with trial guidelines. All medication was administered with food and either directly observed or observed through video by treatment supporters.

Efficacy and safety monitoring was conducted at least every 4 weeks for the first 24 weeks and then at least every 8 weeks for the subsequent 84 weeks. Efficacy was monitored through clinical evaluation and sputum smear and culture.[2] Chest radiography was conducted at baseline and at week 24. Safety was monitored through electrocardiograms, audiometry, blood chemistry analysis, and regular eye and physical examinations. The full investigational schedule has previously been described.[2]

Participants were followed up to week 108 (or to at least week 72 if censored). Those who reached an endpoint continued to be followed up to week 108 for safety. Serious adverse events, adverse events of special interest, pregnancy, and overdoses were reported as part of pharmacovigilance in line with Good Clinical Practice.

Outcomes

Outcomes from stage one of the trial were assessed at 8 weeks after randomisation and have been described and reported previously.[3] In stage two of the trial, the primary outcome was an unfavourable status (a composite of death, treatment failure, treatment discontinuation, recurrence of tuberculosis, or loss to follow-up) at 72 weeks after randomisation. The criterion for an outcome of recurrence was a participant who completed treatment without treatment failure and who had subsequently been diagnosed with and required

treatment for multidrug-resistant tuberculosis. Genetic sequencing was planned to differentiate between disease relapse and re-infection but, owing to technical challenges, has not been completed at the time of publication. Outcomes were assigned by investigators and verified centrally. Uncertain outcomes were referred to an independent outcome adjudication committee.

The prespecified secondary efficacy outcomes were composite unfavourable outcomes at 24 weeks (death, treatment failure, or treatment discontinuation) and 108 weeks (death, treatment failure, treatment discontinuation, recurrence of tuberculosis, loss to follow-up, or still receiving treatment at 108 weeks) after randomisation. Other secondary outcomes were culture conversion at 12 weeks, time to culture conversion, and recurrence of tuberculosis by week 48 post-randomisation (in the investigational groups only). Planned subgroup analyses included age, sex, country of enrolment, fluoroquinolone resistance status, bedaquiline resistance status, HIV status, smoking status, and disease severity. Recruitment before and after the declaration of COVID-19 as a public health emergency was added as an additional subgroup analysis. Other planned analyses, including sensitivity analyses and listing of deaths, were conducted according to the statistical analysis plan (appendix pp 19–49).

The safety outcomes in stage two of the trial were a composite of one or more adverse events of grade 3 or higher or serious adverse events at the end of treatment (plus a 30-day window), at 72 weeks, and at 108 weeks following randomisation, and prolongation of the QTcF interval at 24 weeks post-randomisation. Adverse events of special interest were also reported.

As post-hoc analyses, the efficacy and safety outcomes were also analysed in the BPaLC and BPaL groups at weeks 24, 48, 72 and 108. The outcomes of crossed-over participants were also reported.

Statistical analysis

The sample size for stage one was based on the number of participants required to detect culture conversion of less than 40% and a percentage of treatment discontinuation for any cause and death of greater than 45% in an investigational group. 60 participants in each group were required to achieve 80% power to reject both null hypotheses. The detailed assumptions have been previously reported.[2]

The sample size calculation for stage two was based on a non-inferiority comparison for a composite unfavourable outcome at 108 weeks (assumed to be 50% in the standard care group and 45% in the investigational groups), a non-inferiority margin of 12%, and a power of 85%. Allowing for both the adaptive nature of the design and the multiple comparisons due to the possible three investigational groups being assessed at the end of stage two, a one-sided type I error of 1·7% was assumed, and 181 participants per group would be required.

The intention-to-treat population was defined as all randomly assigned participants who were dispensed study medication on at least one occasion, with participants analysed in the study group to which they were allocated. The modified intention-to-treat population, in which the primary outcome was analysed, included all randomly assigned participants who were dispensed study medication on at least one occasion and had evidence of resistance to at least rifampicin; the tests conducted were dependent on the protocol version under which the participant was enrolled. Participants who switched from standard care to BPaLM after enrolment was stopped on March 18, 2021, were excluded from the modified intention-to-treat population for the main analysis. Participants were analysed on the basis of the group to which they were allocated at enrolment. The per-protocol population was a subset of the modified intention-to-treat population and excluded participants who did not complete a protocol-adherent course of treatment (other than because of treatment failure or death) and participants who discontinued treatment early because they violated at least one of the inclusion or exclusion criteria. A planned sensitivity analysis of the modified intention-to-treat population including participants who switched from standard care to BPaLM after enrolment was also conducted. The safety population was defined as the intention-to-treat population but with participants analysed according to the regimen received. All safety analyses were conducted on the safety population. For crossed-over participants, the safety analyses also excluded any events that occurred after the time at which participants switched groups.

The primary efficacy and safety comparisons assumed a two-sided 96·6% CI for investigational groups assessed in stage two. For binary outcomes we report the absolute difference in the percentages of participants experiencing the outcome using a generalised linear model for a binomial outcome with an identity link function. Adjusting for site was planned as a fixed effect in the regression model, although was changed post-hoc to use the Cochran-Mantel-Haenszel approach owing to non-convergence issues. All secondary efficacy outcomes were reported with corresponding two-sided 95% CIs. Time to unfavourable outcome by 72 weeks was summarised using Kaplan-Meier curves. Post-hoc analyses of all stage 2 primary and secondary outcomes between standard care and investigational groups that did not continue after stage one are also presented, with two-sided 95% CIs. Statistical analyses were conducted using Stata (version 16 or later). The margin used to establish non-inferiority was 12%. A between-group difference of at least 3 SD in the interim analysis of a major endpoint was needed to justify stopping or modifying the study prematurely. The trial was overseen by an independent data safety monitoring board, and is registered at ClinicalTrials.gov, NCT02589782.

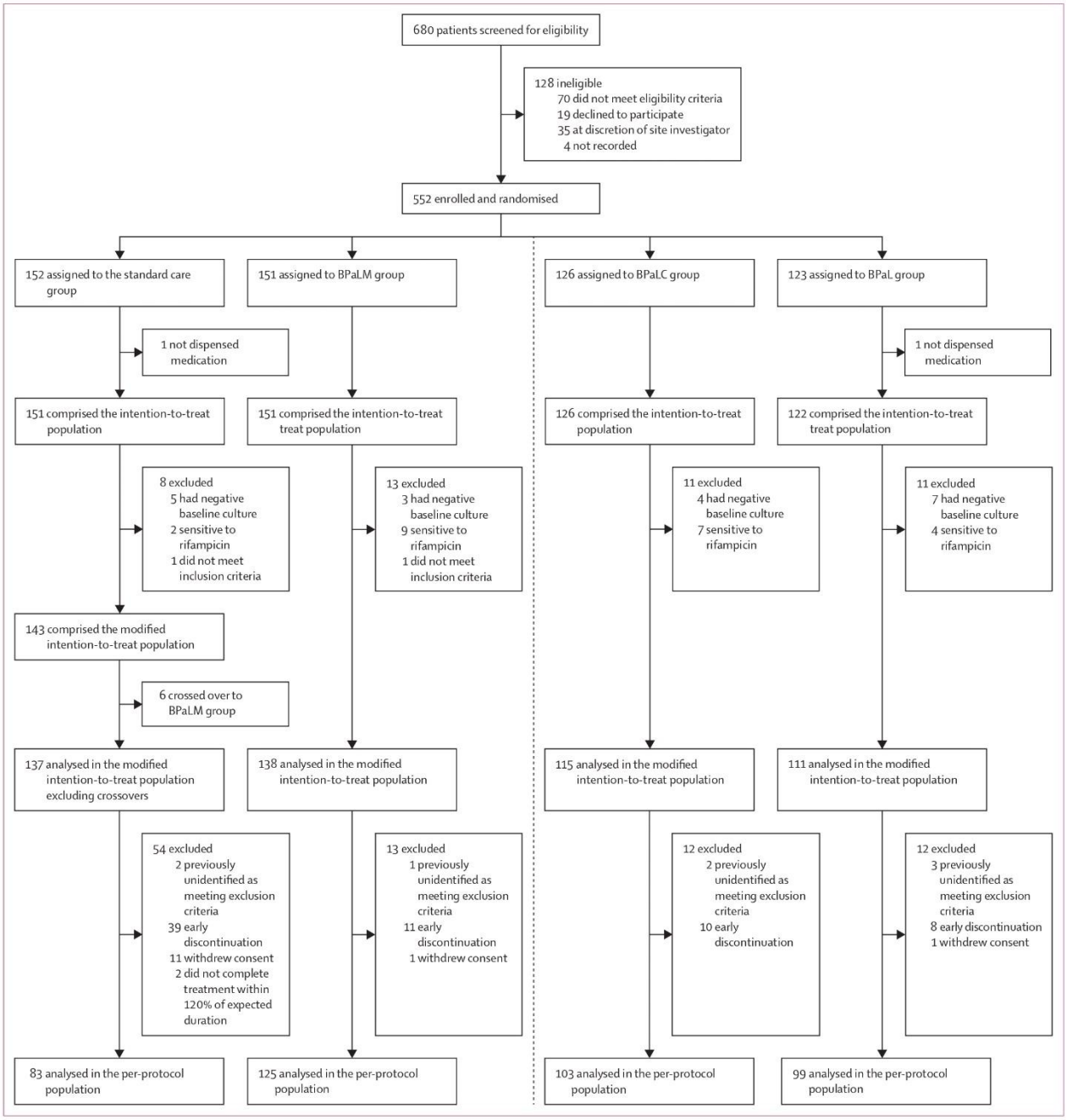


Figure 1: **Trial profile**
The groups to the left-hand side of the dashed line are the standard care and BPaLM groups, which were included in stage two of the study. To the right of the dashed line are the BPaLC and BPaL groups, which discontinued recruitment after transition to stage two. BPaL=bedaquiline, pretomanid, and linezolid. BPaLC=BPaL plus clofamizine. BPaLM=BPaL plus moxifloxacin.

	Standard care (n=143)	BPaLM (n=138)	BPaLC (n=115)	BPaL (n=111)
Country of enrolment				
Belarus	29 (21%)	26 (19%)	19 (17%)	20 (18%)
South Africa	49 (34%)	49 (36%)	43 (37%)	41 (37%)
Uzbekistan	65 (46%)	63 (46%)	53 (46%)	50 (45%)
Age, years	37 (30–46)	35 (27–45)	32 (25–40)	34 (27–44)
Sex				
Female	54 (38%)	61 (44%)	39 (34%)	54 (49%)
Male	89 (62%)	77 (56%)	76 (66%)	57 (51%)
BMI, kg/m²	19·9 (17·5–22·8)	19·7 (17·7–22·7)	19·4 (17·6–22·1)	20·0 (18·1–22·5)
HIV status				
HIV negative	104 (73%)	104 (75%)	84 (73%)	75 (68%)
HIV-positive	39 (27%)	34 (25%)	31 (27%)	36 (32%)
CD4 count, cells per µL	250 (143–445)	330 (223–547)	297 (115–511)	383 (161–550)
CD4 count missing	2 (5%)	2 (6%)	1 (3%)	1 (3%)
Sputum smear				
Smear-positive	94 (66%)	86 (62%)	79 (69%)	73 (66%)
Smear-negative	49 (34%)	52 (38%)	36 (31%)	38 (34%)
Pulmonary cavities				
Present	90 (63%)	76 (55%)	74 (64%)	68 (61%)
Absent	53 (37%)	62 (45%)	41 (36%)	43 (39%)
Fluoroquinolone sensitivity status				
Resistant	32 (22%)	32 (23%)	22 (19%)	25 (23%)
Sensitive	95 (66%)	92 (67%)	87 (76%)	73 (66%)
Resistance status missing	16 (12%)	14 (10%)	6 (5%)	13 (12%)
Bedaquiline sensitivity status				
Resistant	1 (1%)	1 (1%)	2 (2%)	1 (1%)
Sensitive	124 (87%)	116 (84%)	104 (90%)	93 (84%)
Resistance status missing	18 (13%)	21 (15%)	9 (8%)	17 (15%)
QTcF interval, ms	400 (19)	399 (19)	395 (18)	399 (19)
Alanine aminotransferase concentration (IU/L)	20 (15–28)	19 (14–28)	17 (14–26)	19 (14–29)
Data missing	2 (1%)	1 (1%)	1 (1%)	0
Liquid culture at baseline				
Positive	127 (89%)	120 (87%)	107 (93%)	96 (86%)
Negative	17 (12%)	18 (13%)	8 (7%)	15 (14%)
Previous treatment for multidrug-resistant tuberculosis	13 (9%)	18 (13%)	12 (10%)	16 (14%)

Data are n (%), median (IQR), or mean (SD) unless otherwise stated. Percentages may not total 100% owing to rounding. BPaL=bedaquiline, linezolid, and pretomanid. BPaLC=BPaL plus clofazimine. BPaLM=BPaL plus moxifloxacin. IU=international units. QTcF=Fridericia-corrected QT.

Table 1: **Baseline characteristics of the modified intention-to-treat population, including crossover participants**

Role of the funding source

The funder of the study was involved in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

From Jan 16, 2017, to March 18, 2021, 680 patients were assessed for eligibility, of whom 552 were randomly assigned to receive standard care (n=152), BPaLM (n=151), BPaLC (n=126), or BPaL (n=123; figure 1). Of the 507 participants comprising the modified intention-to-treat population, 208 (41%) were female and 299 (59%) were male, the median age was 35 years (IQR 27–43), 140 (28%) were living with HIV (median CD4 count 319 cells per µL [IQR 156–512]), 332 (65%) had smear-positive tuberculosis, 308 (61%) had tuberculosis cavities, and 450 (89%) had culture-positive tuberculosis. A higher proportion of participants had cavitary disease in the BPaLC and standard care groups than in the BpaLM and BpaL groups; markers of disease severity were otherwise similar across groups (table 1). The characteristics of the whole trial population were generally similar (appendix p 6).

179 participants met the criteria for inclusion in the stage one intention-to-treat population (60 in the BPaLM group, 60 in the BPaLC group, and 59 in the BPaL group) and the results have been previously reported.[4] All groups met the eligibility criteria to proceed to stage two. The two groups with higher culture conversion rates, BPaLC and BPaLM, were recommended by the scientific advisory committee to progress. However, owing to recruitment delays and on March 4, 2020, 1 week before COVID-19 was declared as a pandemic, the trial steering committee—in consultation with the scientific advisory committee and the data safety monitoring board—decided to progress only one group to ensure a faster completion of the trial. The BPaLM group was chosen on the basis of higher culture-conversion rates at 8 weeks (BPaLM 77%, BPaLC 67%, and BPaL 46%),[3] lower regimen cost (the prices of clofazimine are higher than those of moxifloxacin), and the classification by WHO of moxifloxacin as a group A drug for tuberculosis; other considerations included the high efficacy of the NiX-TB regimen in quinolone-resistant tuberculosis and, to a lesser extent, concerns surrounding the adverse event profile of clofazimine (such as skin discolouration) as well as its potential cross-resistance with bedaquiline.

On the recommendation of the data safety monitoring board, the trial was stopped for benefit on March 18, 2021, after an unplanned analysis, conducted by request of the board, was found to meet the pre-specified stopping rules. 302 participants met the criteria for inclusion in the stage two intention-to-treat population (and the safety population): 151 in the standard care group and 151 in the BPaLM group. 275 participants were included in the modified intention-to-treat population (137 in the standard care group and 138 in the BPaLM group) and 208 were included in the per-protocol population (83 in the standard care group and 125 in the BPaLM group). Six participants in the standard care group switched to the BPaLM group after enrolment was terminated, and these participants were not included in the primary analysis (figure 1).

Regarding the primary outcome at 72 weeks among the modified intention-to-treat population, 56 (41%) of 137 participants in the standard care group and 16 (12%)

	Modified intention-to-treat population				Per-protocol population (primary analysis)	
	Primary analysis		Post-hoc analysis		Standard care	BPaLM
	Standard care	BPaLM	BPaLC	BPaL		
Number of participants	137	138	115	111	83	125
Number with no unfavourable outcome	81 (59%)	121 (88%)	88 (77%)	96 (86%)	77 (93%)	120 (96%)
Number with an unfavourable outcome	56 (41%)	16 (12%)	27 (23%)	15 (14%)	6 (7%)	5 (4%)
Number non-assessable	0	1 (1%)	0	0	0	0
Unadjusted risk difference*	..	−29·2% (−39·8% to −18·6%)	−17·4% (−28·7% to −6·1%)	−27·4% (−37·8% to −17·0%)	..	−3·2% (−10·3% to 3·9%)
Non-inferiority p value (margin 12%)	..	<0·0001	<0·0001	<0·0001	..	<0·0001
Superiority p value	..	<0·0001	0·0026	<0·0001	..	0·24
Unadjusted risk ratio*	..	0·29 (0·17 to 0·49)	0·57 (0·39 to 0·85)	0·33 (0·20 to 0·55)	..	0·55 (0·16 to 1·93)
Deaths	5 (4%)	0	1 (1%)	1 (1%)	5 (6%)	0
Early discontinuation	50 (37%)	11 (8%)	11 (10%)	11 (10%)	0	0
Adherence issues	11 (8%)	1 (1%)	4 (3%)	3 (3%)	..	..
Adverse event	23 (17%)	7 (5%)	6 (5%)	5 (5%)	..	..
Not meeting inclusion or meeting exclusion criteria†	2 (1%)	1 (1%)	1 (1%)	2 (2%)	..	..
Withdrew consent during treatment	11 (8%)	1 (1%)	0	1 (1%)	..	..
Other	3 (2%)	1 (1%)	0	0	..	..
Treatment failure	0	0	1 (1%)	0	0	0
Lost to follow-up at 72 weeks	1 (1%)	4 (3%)	9 (8%)	0	1 (1%)	4 (3%)
Lost to follow-up	1 (1%)	1 (1%)	6 (5%)	0	1 (1%)	1 (1%)
Withdrew consent	0	3 (2%)	3 (3%)	0	0	3 (2%)
Disease recurrence	0	1 (1%)	5 (4%)	3 (3%)	0	1 (1%)

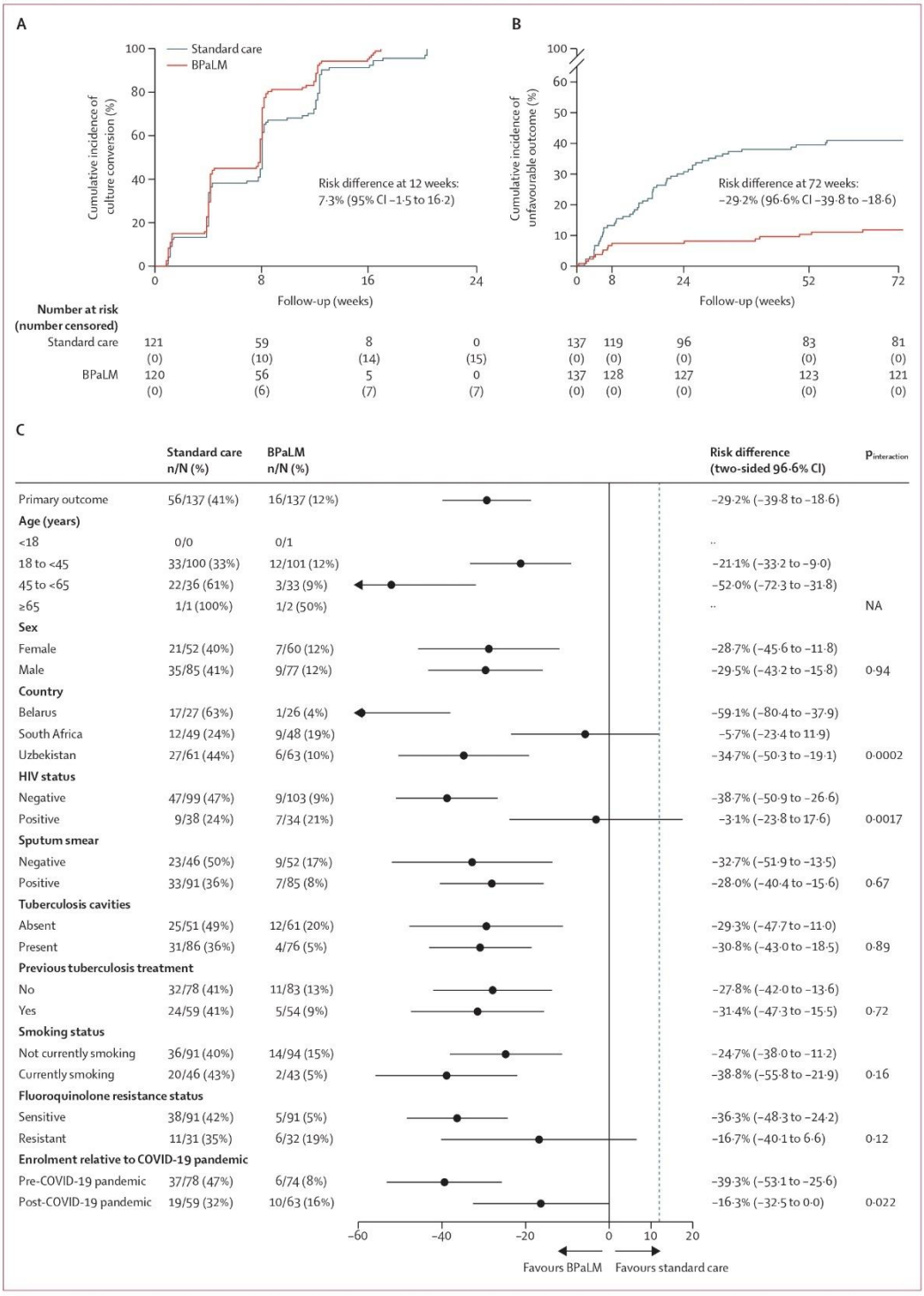
Data are n or n (%) unless otherwise stated. BPaL=bedaquiline, linezolid, and pretomanid. BPaLC=BPaL plus clofazimine. BPaLM=BPaL plus moxifloxacin. *Two-sided 96·6% CI for primary analyses and two-sided 95% CI for post-hoc analysis. Owing to convergence issues, adjusted analyses were conducted using the Cochran-Mantel-Haenszel approach and are reported in the appendix (p 13). †Established after the first dose had been administered.

Table 2: **Primary and post-hoc analyses in the modified intention-to-treat population and primary analyses in the per-protocol population 72 weeks after randomisation**

of 137 participants in the BPaLM group met criteria for the unfavourable outcome (unadjusted risk difference −29·2 percentage points [96·6% CI −39·8 to −18·6]; non-inferiority and superiority p<0·0001; one participant in the BPaLM group had a drug-susceptible disease recurrence and was therefore considered non-assessable). The main reason for meeting the unfavourable outcome definition was early discontinuation (50 [89%] of 56 participants with unfavourable outcomes in the standard care group and 11 [69%] of 16 in the BPaLM group), which was mainly attributed to adverse events (23 [46%] in the standard-care group and seven [64%] in the BPaLM group; table 2). The difference in the risk of an unfavourable outcome between BPaLM and standard care varied depending on country of enrolment or HIV status, and was less pronounced in South Africa (risk difference −59·1 percentage points [96·6% CI −80·4 to −37·9] in Belarus, −5·7 percentage points [−23·4 to 11·9] in South Africa, and −34·7 percentage points [−50·3 to −19·1] in Uzbekistan; $p_{interaction}$=0·0002) and for people living with HIV (risk difference −38·7 percentage points [96·6% CI −50·9 to −26·6] for HIV-negative status and −3·1 percentage points [−23·8 to 17·6] for HIV-positive status; $p_{interaction}$=0·0017; figure 2C).

In the per-protocol population, six (7%) of 83 participants in the standard care group and five (4%) of 125 participants in the BPaLM group met the criteria for the unfavourable outcome, giving an unadjusted risk difference of −3·2 percentage points with the upper CI bound of less than 12% (96·6% CI −10·3 to 3·9; $p_{non\text{-}inferiority}$<0·0001).

Adjustment using the Cochran-Mantel-Haenszel approach and sensitivity analyses were also conducted on



	Standard care n/N (%)	BPaLM n/N (%)	Risk difference (two-sided 96·6% CI)	$p_{interaction}$
Primary outcome	56/137 (41%)	16/137 (12%)	−29·2% (−39·8 to −18·6)	
Age (years)				
<18	0/0	0/1	..	
18 to <45	33/100 (33%)	12/101 (12%)	−21·1% (−33·2 to −9·0)	
45 to <65	22/36 (61%)	3/33 (9%)	−52·0% (−72·3 to −31·8)	
≥65	1/1 (100%)	1/2 (50%)	..	NA
Sex				
Female	21/52 (40%)	7/60 (12%)	−28·7% (−45·6 to −11·8)	
Male	35/85 (41%)	9/77 (12%)	−29·5% (−43·2 to −15·8)	0·94
Country				
Belarus	17/27 (63%)	1/26 (4%)	−59·1% (−80·4 to −37·9)	
South Africa	12/49 (24%)	9/48 (19%)	−5·7% (−23·4 to 11·9)	
Uzbekistan	27/61 (44%)	6/63 (10%)	−34·7% (−50·3 to −19·1)	0·0002
HIV status				
Negative	47/99 (47%)	9/103 (9%)	−38·7% (−50·9 to −26·6)	
Positive	9/38 (24%)	7/34 (21%)	−3·1% (−23·8 to 17·6)	0·0017
Sputum smear				
Negative	23/46 (50%)	9/52 (17%)	−32·7% (−51·9 to −13·5)	
Positive	33/91 (36%)	7/85 (8%)	−28·0% (−40·4 to −15·6)	0·67
Tuberculosis cavities				
Absent	25/51 (49%)	12/61 (20%)	−29·3% (−47·7 to −11·0)	
Present	31/86 (36%)	4/76 (5%)	−30·8% (−43·0 to −18·5)	0·89
Previous tuberculosis treatment				
No	32/78 (41%)	11/83 (13%)	−27·8% (−42·0 to −13·6)	
Yes	24/59 (41%)	5/54 (9%)	−31·4% (−47·3 to −15·5)	0·72
Smoking status				
Not currently smoking	36/91 (40%)	14/94 (15%)	−24·7% (−38·0 to −11·2)	
Currently smoking	20/46 (43%)	2/43 (5%)	−38·8% (−55·8 to −21·9)	0·16
Fluoroquinolone resistance status				
Sensitive	38/91 (42%)	5/91 (5%)	−36·3% (−48·3 to −24·2)	
Resistant	11/31 (35%)	6/32 (19%)	−16·7% (−40·1 to 6·6)	0·12
Enrolment relative to COVID-19 pandemic				
Pre-COVID-19 pandemic	37/78 (47%)	6/74 (8%)	−39·3% (−53·1 to −25·6)	
Post-COVID-19 pandemic	19/59 (32%)	10/63 (16%)	−16·3% (−32·5 to 0·0)	0·022

Figure 2: **Primary composite outcome, culture conversion, and subgroup analysis of the modified intention-to-treat population at week 72**
(A) Kaplan-Meier plot for culture conversion in the modified intention-to-treat populations of the BPaLM and standard care groups. (B) Kaplan-Meier estimates of the time to an unfavourable outcome by week 72 in the modified intention-to-treat populations of the BPaLM and standard care groups. No patients are censored because deaths, withdrawals, and losses to follow-up are all part of the composite outcome. (C) Forest plot of the risk difference in the prespecified subgroup analyses between the standard care and BPaLM regimens, analysed at week 72 in the modified intention-to-treat population. Dashed vertical line shows the non-inferiority margin at 12%. BPaLM=bedaquiline, pretomanid, and linezolid plus moxifloxacin. NA=not applicable. Pre-COVID-19 pandemic is defined as the period before Jan 30, 2020, when COVID-19 was declared as a Public Health Emergency of International Concern by WHO; post-COVID-19 pandemic is defined as Jan 30, 2020 onwards.

the primary outcome. For all comparisons between study groups, the adjusted risk differences were consistent with the unadjusted effects (appendix p 13), as was the sensitivity analysis based on the modified intention-to-treat population that included the six participants in the standard care group who switched treatment (appendix pp 14–15). A sensitivity analysis excluding participants who were recruited before the implementation of the 2019 WHO guidelines[3] for standard care showed that non-inferiority was maintained (risk difference –19·1 percentage points [95% CI –30·9 to –7·3]; appendix p 14).

Results for the unfavourable outcome at 108 weeks in the modified intention-to-treat population were consistent with those for the primary outcome (appendix p 10). Two disease recurrences had occurred by 108 weeks: one in the standard care group and one in the BPaLM group. In the per-protocol population, the unadjusted risk difference for BPaLM versus standard care was larger at 108 weeks (–10·1 percentage points [95% CI –18·9 to –1·3]) than at 72 weeks (–3·2 percentage points [–10·3 to 3·9]), mostly driven by the number of deaths in the standard-care group (zero in the BPaLM group *vs* six in the standard care group; appendix p 16). Of the deaths, four were considered related to treatment (sudden cardiac death, sudden death, acute pancreatitis, and suicide) and two were not (stab wound and COVID-19 pneumonia). Study group effects on unfavourable outcomes (death, treatment failure, and treatment discontinuation) at 24 weeks in the modified intention-to-treat population were consistent with the 72-week and 108-week outcomes (appendix p 11).

Culture conversion at 12 weeks was observed for 99 (82%) of 121 patients for whom conversion could be defined in the standard care group and 107 (89%) of 120 patients in the BPaLM group (risk difference 7·3 percentage points [95% CI –1·5 to 16·2]; figure 2A). Median time to culture conversion was 56 days (IQR 28 to 83) in the standard care group and 55 days (28 to 57) in the BPaLM group (unadjusted hazard ratio 1·38 [95% CI 1·05 to 1·81]; appendix p 12).

A post-hoc evaluation of long-term outcomes was conducted in the BPaLC and BPaL groups. By week 72, in the modified intention-to-treat population and compared with standard care, unadjusted risk differences were –17·4 percentage points (95% CI –28·7 to –6·1) for BPaLC and –27·4 percentage points (–37·8 to –17·0) for BPaL, indicating non-inferiority (table 2). Non-inferiority was also shown in the per-protocol population for BPaL (–3·2% [–10·0 to 3·6]) but not for BPaLC (8·3% [–0·1 to 17·2]; appendix p 7).

By week 108 after randomisation, the effect estimates remained similar to those measured at week 72: in the

	Standard care (n=151)	BPaLM (n=151)	BPaLC (n=126)	BPaL (n=122)
QTcF interval at 24 weeks				
Number with QTcF interval measured	96	128	101	99
Mean QTcF interval, ms	440·9	425·1	436·3	421·8
Mean difference *vs* standard care, ms*	..	–17·5 (–22·0 to –12·9)	–4·4 (–8·8 to –0·1)	–21·1 (–25·6 to –16·6)
Grade ≥3 adverse effects or serious adverse effects during or within 30 days after treatment				
Participants with at least one event	71 (47%)	26 (17%)	31 (25%)	26 (21%)
Number of events	118	40	42	33
Serious†	46	10	16	12
Grade ≥3†	107	39	41	29
Risk difference *vs* standard care, percentage points‡	..	–29·8 (–40·6 to –19·0)	–22·4 (–33·4 to –11·5)	–25·7 (–36·5 to –14·9)
Grade ≥3 adverse effects or serious adverse effects within 108 weeks				
Participants with at least one event	75 (50%)	35 (23%)	40 (32%)	30 (25%)
Number of events	127	58	54	51
Serious†	53	13	26	22
Grade ≥3†	116	56	52	47
Risk difference *vs* standard care, percentage points‡	..	–26·5 (–37·8 to –15·2)	–17·9 (–29·3 to –6·5)	–25·1 (–36·1 to –14·0)
Grade ≥3 adverse effects or serious adverse effects within 72 weeks				
Participants with at least one event	72 (48%)	34 (23%)	38 (30%)	29 (24%)
Number of events	121	53	52	45
Serious†	48	13	24	20
Grade ≥3†	110	51	50	41
Risk difference *vs* standard care, percentage points‡	..	–25·2 (–36·4 to –13·9)	–17·5 (–28·8 to –6·2)	–23·9 (–34·9 to –12·9)

Data are n, n (%), mean, mean difference (CI), or risk difference (CI). CIs are 96·6% for BPaLM *vs* standard care comparisons and 95% for BPaLC *vs* standard care and BPaL *vs* standard care comparisons. BPaL=bedaquiline, linezolid, and pretomanid. BPaLC=BPaL plus clofazimine. BPaLM=BPaL plus moxifloxacin. QTcF=Fridericia-corrected QT. *Adjusted for site and baseline QTcF interval. †Not mutually exclusive. ‡Unadjusted for site.

Table 3: **Safety outcomes in the safety population**

modified intention-to-treat population, BPaLC remained non-inferior to standard care at week 108 (appendix p 9). Of note, disease recurrence occurred in five (4%) of 115 participants in the BPaLC group, four (4%) of 111 in the BPaL group (appendix p 9). New resistance to bedaquiline was observed in three of four isolates from participants with disease recurrence, all in the BPaL group; of these, an isolate from one participant also showed resistance to clofazimine (appendix p 13).

Among the safety population, 72 (48%) of 151 participants in the standard care group had at least one adverse event of grade 3 or higher or serious adverse events within 72 weeks (121 events in total), compared with 34 (23%) of 151 participants in the BPaLM group (53 events; risk difference −25·2 percentage points [96·6% CI −36·4 to −13·9]; table 3). Common adverse events included hepatic disorders (22 events in 15 (10%) participants receiving standard care *vs* 17 events in 12 (8%) participants receiving BPaLM); cardiac disorders (19 *vs* two), most of which were due to QT-prolongation; and anaemia (13 *vs* six; appendix pp 16–18). Compared with the BPaLM group, the proportions of participants with adverse events of grade 3 or higher or serious adverse events was similar in the BPaL group (29 [24%] of 122 participants; 45 events) and higher in the BPaLC group (38 [30%] of 126 participants; 52 events). Similar results were found when assessing adverse events over 108 weeks (table 3). Mean Fridericia-corrected QT (QTcF) intervals at 24 weeks were 440·9 ms in the standard care group and 425·1 ms in the BPaLM group (mean difference −17·5 ms [96·6% CI −22·0 to −12·9]). Mean QTcF intervals were 436·3 ms in the BPaLC group and 421·8 ms in the BPaL group (table 3). Nine participants died by week 108: six (4%) in the standard care group, zero in the BPaLM group, one (1%) in the BPaLC group (chronic obstructive pulmonary disease; unrelated to treatment), and two (2%) in the BPaL group (seizure and lower respiratory tract infection; unrelated to treatment).

Discussion

This study corroborates, with increased precision, the findings from the interim analysis of the TB-PRACTECAL trial that a 24-week oral regimen consisting of bedaquiline, pretomanid, linezolid, and moxifloxacin is non-inferior to standard care for the treatment of patients with pulmonary rifampicin-resistant tuberculosis.[4] In post-hoc analyses, BPaLC and BPaL were also shown to be non-inferior to standard care. The BPaLM, BPaLC, and BPaL groups had fewer serious adverse events and adverse events of at least grade 3 than the standard care group. To our knowledge, this study is the first randomised controlled trial to examine BPaL-based regimens for rifampicin-resistant tuberculosis. The study provides robust, generalisable data showing efficacy among similar numbers of male and female participants from three countries, and is inclusive of people with HIV coinfection and severe rifampicin-resistant disease with and without fluoroquinolone resistance; as such, the participants are broadly representative of adult and adolescent patients with rifampicin-resistant tuberculosis worldwide.

The effect estimate for the primary outcome comparing BPaLM versus standard care at 72 weeks was smaller in this final analysis (risk difference −29·2 percentage points) than in the interim findings (−37·2 percentage points).[3] This difference can be mostly explained by the better performance of the standard care group in the final analysis,[3] which is possibly due to improvements in standard care throughout the trial. In 2019, the update to the WHO consolidated guidelines on drug-resistant tuberculosis treatment prioritised the addition of bedaquiline and linezolid to most regimens, withdrew the use of second-line injectable agents, and allowed shorter regimens of 9–12 months duration.

In secondary and post-hoc analyses, culture conversion was faster in the BPaLM group than in the standard care group, and fastest in BPaLM among all three investigational groups (appendix p 12). Deaths were uncommon in the investigational groups; three deaths occurred among all three investigational groups compared with six in the standard care group by week 108 (appendix p 10). Despite disruptions due to the COVID-19 pandemic, BPaLM maintained high efficacy in participants recruited after WHO's declaration of the disease as a Public Health Emergency of International Concern on Jan 30, 2020.

The subgroup analyses showed that all risk difference point estimates favoured BPaLM over standard care at 72 weeks post-randomisation in the modified intention-to-treat population, including by sex, age, disease severity, re-treatment status, and smoking status. The upper bounds of the CIs were greater than zero but within the 12% non-inferiority margin in participants with baseline fluoroquinolone resistance and in those who were enrolled in South Africa; having HIV at baseline, however, resulted in a upper bound higher than the 12% non-inferiority margin. We note a significant interaction ($p<0·05$) between the BPaLM and standard care groups for country of enrolment, HIV status, and for those enrolled after the declaration of COVID-19 as a public health emergency on Jan 30, 2020. Almost all participants who were HIV-positive were enrolled in South Africa (127 [91%] of 139); however, whether the interaction was driven by the location of participants or their HIV status is difficult to establish. The standard of care performed better in HIV-positive patients than in HIV-negative patients, which was unexpected. Further elucidation of these potential interactions in real-world settings is warranted.

Our data are consistent with those from other studies showing that BPaL-based regimens are associated with around 7–16% unsuccessful outcomes.[5,6] A network meta-analysis was conducted to inform WHO guideline development. This analysis included the interim TB-PRACTECAL data (participants with outcomes up to March 18, 2021). The BPaL regimen was shown to have

higher efficacy than standard care regimens (relative risk of treatment success 1·32 (95% CI 1·19–1·39) for the 18–20-month, all-oral regimen, 1·45 (1·38–1·52) for the WHO 9–11-month short regimen, and 1·52 (1·38–1·55) for the South African 9–11-month short regimen). A 600 mg dose of linezolid for 26 weeks was found to have similar efficacy to a 1200 mg dose but with fewer grade 3–5 adverse events (six [14%] of 43 patients with 600 mg *vs* eight [19%] of 44 patients with 1200 mg) at 12 months after randomisation. Finally, the network meta-analysis found successful outcomes in 55 (89%) of 62 patients treated with BPaLM compared with 46 (77%) of 60 of those treated with BPaL (absolute risk reduction 1·15 [95% CI 0·95–1.38]).[4] This difference is more pronounced than the absolute outcomes found in this final analysis. However, other considerations—such as time to culture conversion, recurrence, and resistance development—would need to be included when deciding on the appropriate regimen to use.

The performance of the standard of care was lower than in trials of rifampicin-resistant or multidrug-resistant tuberculosis reported in the past 4 years (STREAM[7] and MDR-END[8]). A very tight limit was set in which participants missing treatment for 2 continuous weeks would be discontinued from the trial. This limit was intended to protect participants in investigational groups in case the barrier to acquired drug resistance was very low. Ultimately, we found that meeting these criteria for continuation was most difficult for participants in the standard care group who were struggling with adverse events or adherence to treatment, and these difficulties led to early discontinuation in a high proportion of participants.

Disease recurrence occurred in one participant in the BPaLM group, five of those in the BPaLC group, and four of those in the BPaL group. New resistance to bedaquiline was observed only in the BPaL group in isolates from three of four recurrences. No other new resistance to bedaquiline, linezolid, or pretomanid was detected among the other nine participants who developed recurrence or treatment failure across the four groups. Analysis of paired genome sequencing results to confirm relapse is ongoing, so some of these recurrences could be due to reinfection. The ZeNix trial, which studied BPaL regimens with different doses of linezolid, reported recurrence in four (2%) of 181 participants.[4,5]

This study has several limitations, including those described previously.[3] We previously acknowledged the indirectness of the analysis, with many participants receiving an outdated standard of care that is no longer recommended. The WHO consolidated guidelines on drug-resistant tuberculosis treatment were revised in March, 2019, and subsequent participants received standard of care in line with these guidelines (appendix p 7). This change to the standard of care is reflected in the updated analysis, in which the majority (95 [69%] of 137) of participants received the then-current standard of care. A sensitivity analysis showed the effect estimate remained at –19·1% (–31·9% to –6·3%) when participants recruited before the 2019 WHO drug-resistant tuberculosis guidelines were implemented were excluded. The heterogeneity in standard of care could have influenced the interaction analysis by country and HIV status.

Additionally, the sponsor, participants, and investigators were made aware that the trial was stopped for efficacy, which could have introduced bias. Six participants who crossed over from the standard care group to the BPaLM group were excluded from the modified intention-to-treat population because the regimen that induced efficacy could not be established (appendix p 15). Sensitivity analyses suggest that the inclusion of these participants would not have changed the effect estimate in a clinically important way (appendix pp 14–15). Three grade 3 adverse events occurred in this group of six participants after switching to BPaLM (appendix p 18). Outcome adjudication was conducted by unmasked investigators, which could also have introduced bias. Difficult cases were assessed by an independent committee masked to the treatment group, when possible.

As a conservative measure, we included loss to follow-up in the composite unfavourable outcome. The smaller effect estimate seen with BPaLC versus standard care was principally driven by participants lost to follow-up and we do not have a hypothesis linked to the treatment allocation that explains this difference. The differences in loss to follow-up across groups had largely resolved by week 108 and could have occurred by chance (appendix p 10). However, the trial was not powered to compare the investigational groups with each other. The inclusion of loss to follow-up as part of composite unfavourable outcomes, as is the case in programmatic classifications, has drawbacks as it is more likely to be an issue of missing data rather than unaccounted-for adverse outcomes. We agree, as others have suggested, that future late-phase tuberculosis trials should reconsider including loss to follow-up as an assessable outcome.[9]

Several outstanding research questions remain. The optimal dose of linezolid remains unknown. A starting dose of 600 mg seems to be the most tolerable; however, the optimal duration of treatment is less clear, as is the role of dose reduction. Ongoing pharmacokinetic studies could assist in answering this question.[10] Some argue that therapeutic drug monitoring could have a role in personalising dosing,[11] but this is unlikely to be accessible in all settings. Whether similar results can be achieved with alternative fluoroquinolones (such as levofloxacin) or with nitroimidazoles (such as delamanid) is unknown, although early results are promising.[12] Newer oxazolidinones could also offer a better safety and tolerability profile than linezolid.[13] Phenotypic drug susceptibility testing breakpoints for pretomanid need to be confirmed and further information is also needed on the performance of the regimen in settings with a high

prevalence of *Mycobacterium tuberculosis* lineage 1.[14] Data are also needed in children, pregnant people, and those with extrapulmonary tuberculosis. The country and HIV-status subgroup findings in our study warrant further investigation, as almost all the participants with HIV were from South Africa. Additionally, South African participants in the standard care group were treated with the 9–11 month shorter oral regimen including linezolid for 8 weeks; this regimen was not in use at other sites during recruitment.

Despite the limitations and outstanding questions, these BPaL-based regimens perform better than the 9–20-month standard of care; they are shorter, have a lower pill burden, improve quality of life, and have been shown to be cost-effective.[15] BPaLM, BPaLC, and BPaL have the potential to improve the outcomes of thousands of people with rifampicin-resistant tuberculosis, and we call on national tuberculosis programmes and partners to accelerate the implementation of these regimens.[16]

Contributors

B-TN, PdC, and DAM conceived the study. B-TN, CB, EK, and IM led the sponsor project management team. MD compiled reports for the data safety monitoring board. NP, ZT, RM, VS, IL, SR, and MR were site principal investigators and were responsible for participant recruitment and data collection. TM oversaw the laboratory set-up and monitoring. MS, DAM, KR, and PdC provided study oversight via the trial steering committee. Statistical analysis was done by MD and KF. The first draft of the manuscript was written by CB, IM, EK, B-TN, KF, and MD. The manuscript was revised, edited, and read by all authors. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication. KF, MD, CB, IM, EK, and B-TN accessed and verified all data in the study.

Declaration of interests

B-TN, CB, EK, and IM are employees of Médecins Sans Frontières. MR participates on the data safety monitoring board for the BEAT-TB trial (NCT04062201). PdC is a former employee of Médecins Sans Frontières and received consultancy fees and conference attendance support from the organisation between 2020 and 2022. He has also received grants from the Department of Foreign Affairs and Trade and the Medical Research Future Fund of the Australian Government; FIND; the Global Fund to Fight AIDS, Tuberculosis and Malaria; and STOP TB; and honoraria for training sessions from the regional Green Light Committee (Western Pacific Regional Office), of which he is an unpaid committee member. TM has received grants from the Global Alliance Against Tuberculosis, the European & Developing Countries Clinical Trials Partnership, and the EU Innovative Medicines Initiative; is co-editor in chief of *Annals of Clinical Microbiology and Antimicrobials* (Springer Nature); and is the chair of the Acid Fast Club (unpaid). MD and KF received salary funding paid to the London School of Hygiene & Tropical Medicine (London, UK) by Médecins Sans Frontières. All other authors declare no competing interests.

Data sharing

De-identified individual patient data and a data dictionary will be made available on one or more scientific data repositories following publication. The study protocol, informed consent form, and statistical plan have previously been made available.[2,3] The sponsor of this trial (Médecins Sans Frontières) intends to make all data publicly available by the end of March, 2024, via TB-PACTS (Critical Path Institute; https://c-path.org/programs/tb-pacts/accessing-tb-pacts/).

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Chapter 3: PRACTECAL-PKPD Methods

3.1. Introduction

The PRACTECAL-PKPD study included the study of all drugs in the investigational regimens in the TB-PRACTECAL trial. These are bedaquiline (B), pretomanid (Pa), linezolid (L), moxifloxacin and clofazimine. All drugs in the PRACTECAL backbone (B,Pa,L) and clofazimine were prioritised for modelling and analysis for the PhD thesis. This methods chapter comprises of a publication of the PRACTECAL-PKPD study protocol and additional detailed methodology common to all drugs. A concise methods section specific to each drug is included in chapters 4, 5, 6 and 7 of the thesis.

3.2. Objectives

Study objectives of the PhD are a subset of the ones defined in the PRACTECAL-PKPD study protocol. After study implementation and bioanalysis of all drugs, the scope is then limited to pharmacokinetic modelling and probability of target attainment for bedaquiline, pretomanid, linezolid and clofazimine. This adjustment in scope was made to facilitate timely release of study results (outside scope of PhD) and timely completion of PhD.

Therefore, the objectives of the pharmacokinetic part of the PhD were:

1. Design and implement a study to measure the plasma concentrations of bedaquiline, pretomanid, linezolid, moxifloxacin and clofazimine in a sub-set of patients in the TB-PRACTECAL trial.
2. Using population pharmacokinetic modelling, estimate PK parameters for bedaquiline, pretomanid, linezolid and clofazimine.
3. Using the derived Pharmacokinetic parameters and minimum inhibitory concentrations (MIC), simulate the probability of target attainment (PTA) for bedaquiline, pretomanid, linezolid and clofazimine.

3.3. The PRACTECAL-PKPD study protocol paper

Nyang'wa BT, Kloprogge F, Moore DAJ, Bustinduy A, Motta I, Berry C, Davies GR. Population pharmacokinetics and pharmacodynamics of investigational regimens' drugs in the TB-PRACTECAL clinical trial (the PRACTECAL-PKPD study): a prospective nested study protocol in a randomised controlled trial. BMJ Open. 2021 Sep 6;11(9):e047185. doi: 10.1136/bmjopen-2020-047185.

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Student ID Number	1700547	**Title**	Dr
First Name(s)	Bern-Thomas		
Surname/Family Name	Nyang'wa		
Thesis Title	Short, effective and safe all-oral treatment for rifampicin resistant tuberculosis: TB-PRACTECAL trial and its drugs pharmacokinetics		
Primary Supervisor	Prof. David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

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Population pharmacokinetics and pharmacodynamics of investigational regimens' drugs in the TB-PRACTECAL clinical trial (the PRACTECAL-PKPD study): a prospective nested study protocol in a randomised controlled trial

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ABSTRACT

Introduction Drug-resistant tuberculosis (TB) remains a global health threat, with little over 50% of patients successfully treated. Novel regimens like the ones being studied in the TB-PRACTECAL trial are urgently needed. Understanding anti-TB drug exposures could explain the success or failure of these trial regimens. We aim to study the relationship between the patients' exposure to anti-TB drugs in TB-PRACTECAL investigational regimens and their treatment outcomes.

Methods and analysis Adults with multidrug-resistant TB randomised to investigational regimens in TB-PRACTECAL will be recruited to a nested pharmacokinetic-pharmacodynamic (PKPD) study. Venous blood samples will be collected at 0, 2 and 23 hours postdose on day 1 and 0, 6.5 and 23 hours postdose during week 8 to quantify drug concentrations in plasma. Trough samples will be collected during week 12, 16, 20 and 24 visits. Opportunistic samples will be collected during weeks 32 and 72. Drug concentrations will be quantified using liquid chromatography-tandem mass spectrometry. Sputum samples will be collected at baseline, monthly to week 24 and then every 2 months to week 108 for MICs and bacillary load quantification. Full blood count, urea and electrolytes, liver function tests, lipase, ECGs and ophthalmology examinations will be conducted at least monthly during treatment.

PK and PKPD models will be developed for each drug with nonlinear mixed effects methods. Optimal dosing will be investigated using Monte-Carlo simulations.

Ethics and dissemination The study has been approved by the Médecins sans Frontières (MSF) Ethics Review Board, the LSHTM Ethics Committee, the Belarus RSPCPT ethics committee and PharmaEthics and the University of Witwatersrand Human Research ethics committee in South Africa. Written informed consent will be obtained from all participants. The study results will be shared with public health authorities, presented at scientific conferences and published in a peer-reviewed journal.

Trial registration number NCT04081077; Pre-results.

Strengths and limitations of this study

- This is the first study that prospectively evaluates the pharmacokinetic (PK) and PK-pharmacodynamic properties of three novel exclusively oral, short course multidrug-resistant-tuberculosis treatments; bedaquiline, pretomanid, linezolid in absence and presence of either moxifloxacin or clofazimine.
- The study is including participants from key populations (HIV positive and on antiretrovirals) as well as in ethnically diverse populations (South Africa and Belarus).
- Being a nested study, the sample size is mainly determined by the parent study.

INTRODUCTION

Tuberculosis (TB) remains the deadliest infectious disease in the world, killing an estimated 1.4 million people of the 10 million people who developed the disease in 2019. TB that is resistant to the most powerful anti-TB drug, rifampicin resistant (RR)-TB, caused disease in half a million people representing 5% of all TB and yet is estimated to have caused death in 15% (182 000).[1] WHO currently recommends use of either a shorter treatment regimen (9–12 months) or a longer regimen lasting up to 20 months for the treatment of multidrug-resistant (MDR)/RR-TB depending on prior exposure or proven resistance to second line anti-TB drugs. A 6–9 months regimen consisting of bedaquiline (B), pretomanid (Pa) and linezolid (Lzd) which was used in the NiX-TB study[2] has been recommended for use in operational research.[3]

PRAgmatic Clinical Trial for a more Effective, Concise And Less toxic MDR-TB

regimens (TB-PRACTECAL) is a multicentre, open label, phase 2–3 randomised controlled trial evaluating 6 months duration, exclusively oral regimens containing bedaquiline, pretomanid, linezolid±moxifloxacin (Mfx) or clofazimine (Cfz) for the treatment of microbiologically confirmed pulmonary RR-TB. In the TB-PRACTECAL trial, B is dosed at 400 mg daily for 2 weeks and then 200 mg three times a week for 22 weeks. Pretomanid is dosed at 200 mg daily for 24 weeks. Linezolid is dosed at 600 mg daily for 16 weeks and then reduced to 300 mg daily for 8 weeks. Mfx is given at 400 mg daily for 24 weeks and Cfz is dosed at 100 mg daily for body weight above 33 kg and 50 mg daily below 33 kg for 24 weeks.

A cumulating body of evidence has shown that anti-TB drug exposure especially in HIV positive patients varies significantly.[4]Moreover, low-drug concentrations are linked to poor outcomes,[5] particularly microbiological failure.[6] Identifying the optimal dose and duration of drugs such as linezolid in treating RR-TB remains a global research priority.[3]

If the TB-PRACTECAL trial identifies successful regimens, the PRACTECAL-pharmacokinetic-pharmacodynamic (PKPD) substudy will provide explanatory evidence to why the tested regimens at the chosen doses and administration scenario are efficacious. And if the regimens have not been shown to be non-inferior, allow the understanding of whether variability of particular drug exposures could have played a part in the efficacy or safety outcomes and make appropriate recommendations for further research.[7]

We; therefore, aim to study the relationship between the patients' exposure to anti-TB drugs in the TB-PRACTECAL trial investigational regimens and their respective treatment outcomes.

METHOD AND ANALYSIS

Study design development

The Fisher Information Matrix (FIM)[8] was used to optimise a venous blood sampling design that supports PK model development in that expected PK model parameter estimate precision will be ≤20%.

Identification of prior information

A structured literature search was done in Medline, Embase and PubMed databases and relevant conferences in August 2017, with the following search terms (population pharmacokinetics AND drug_name) to identify published population PK models. The search yielded 5, 0, 3, 1 and 5 relevant papers for linezolid,[9–13] pretomanid, bedaquiline,[14] Cfz[15] and Mfx,[16–20] respectively. Authors were contacted when full text articles were not accessible and drug developers were contacted with the request to share unpublished models.[21] Where more than one suitable PK model was available, the following hierarchically listed criteria were used to select a suitable model to be used for design optimisation: study population (MDR-TB, TB, non-TB patients or healthy subjects), original PK study sample size (larger sample sizes preferred), and a critical appraisal of the publications including whether the authors reported enough parameters to allow for parameterisation of the model.

Identification and definition of constraints

The FIM was maximised given a series of design constrains. First, samples could only be collected on scheduled visits with planned laboratory sample collection as per main study protocol. Second, venous plasma samples could only be scheduled during day nurse working and laboratory opening hours in order to warrant access to staff, centrifuges and freezers. Lastly, the sampling intervals could not be shorter than 15 min in order to warrant that the protocol is executable by clinical and laboratory staff.

Sampling schedule optimisation

ED design optimisation with uncertainty on clearance estimates, using PopED; an R-package[22] (V.0.3.2), was used to simultaneously optimise a venous blood sampling schedule for pretomanid[21] and linezolid[13] in first instance. Subsequently, bedaquiline,[23] Cfz[15] and Mfx[19] expected elimination clearance estimates were evaluated given the optimal venous plasma sampling designs for pretomanid and linezolid. The two-step approach was chosen due to the distinctly different PK profiles of bedaquiline, Cfz and Mfx, with an elimination phase outside the 24-hour dosing schedule, when compared with pretomanid and linezolid.

Design evaluation

The optimal venous blood sampling schedule was subsequently evaluated for each study drug using the stochastic simulation and estimation (SSE) function of PsN[24] with NONMEM (V.7.3). The optimal evaluated design was for 240 patients sampled at day 0 (0, 2, 23 hours), week 8 (0, 6.5, 23 hours), trough at weeks 12, 16, 20 and 24 and opportunistic at week 32 and 72. The expected relative SEs (RSEs) for clearance were below 20% for each drug (table 1).

Study design

Main study question

What is the relationship between the patients' exposure to anti-TB drugs in the TB-PRACTECAL trial investigational regimens and their respective treatment outcomes?

Primary objective

Measure the plasma concentrations of pretomanid, linezolid, bedaquiline, Cfz and Mfx in a subset of patients in the TB-PRACTECAL trial and using population PK models, estimate the population exposure metrics (Cmin, Cmean, Cmax, area under the curve (AUC)) for the individual drugs in the TB-PRACTECAL trial.

Secondary objectives

1. Develop PK models for each of the study drugs.
2. Develop a PKPD model to characterise the relationship between drug exposure, baseline clinical covari-

Table 1 Expected clearance RSEs per drug, using the chosen sampling schedule

Drug	Simulated clearance (mL/min)	Mean re-estimated (mL/min)	RSE re-estimated (%)
Linezolid	1.86	1.96	16.6
Clofazimine	10	11.2	6.32
Pretomanid	7.71	4.16	2.02
Bedaquiline	2.78	2.84	6.08
Moxifloxacin	10.6	12.6	1.47*

*The moxifloxacin PK model used an informative prior from literature in the SSE.
PK, pharmacokinetic; RSE, relative SE; SSE, stochastic simulation and estimation.

ates, baseline minimum inhibitory concentrations and early bactericidal effect.
3. Study correlations between baseline clinical covariates, baseline minimum inhibitory concentrations, drug exposure and longitudinal PKPKD markers and long-term treatment outcome defined as success at end of treatment and remaining relapse free for 1 year after successful treatment.
4. Develop PKPD models investigating associations between PK parameters and treatment emergent toxicity.
5. Use results from the aforementioned algorithms to develop a hypothesis on the optimal dosing of linezolid and Cfz using Monte-Carlo simulations.

Patient and public involvement

Patients were not directly involved in the design of this study. However, the parent clinical trial engaged patients in the setup and implementation.[25]

Study setting

The study is recruiting and being implemented in five hospitals (figure 1) in Belarus and South Africa. The drug quantification bioanalysis will be conducted at the University of Liverpool, Liverpool, UK and the mycobacteriology is done at Republican Specialised Practical Centre for Pulmonology and Tuberculosis National Reference Laboratory in Minsk for Belarus samples and Cytespace Africa laboratories in Pretoria, South Africa.

Study population

We are seeking to describe the population drug exposures and their variability in the target population, so a traditional power calculation was not done. The total number of patients recruited into the study is driven by the timing of starting the substudy and proportion of patients consenting. Based on the optimal design parameters, we aimed to recruit a maximum of 240 patients resulting

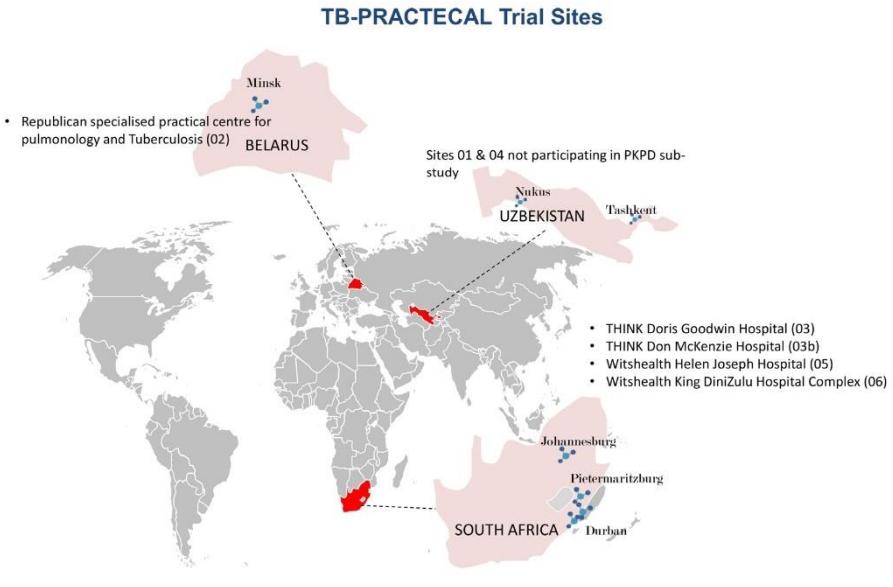

Figure 1 Trial sites participating in the PRACTECAL-PKPD sub-study. PKPD, pharmacokinetic-pharmacodynamic; TB, tuberculosis.

in just under 3000 drug concentration observations. We recruited 97 patients with an expected 1164 samples to be available for bioanalysis and at least 3492 drug concentration measurements for the four study drugs.

Inclusion/exclusion criteria

All adult patients recruited into the investigational arms of the parent TB-PRACTECAL trial in the approved sites are eligible to join the study (https://clinicaltrials.gov/ct2/show/NCT02589782) with the following eligibility criteria:

Inclusion criteria:

Patients eligible for inclusion in the trial must fulfil all of the following criteria:

- Male or female subjects aged 15 years of age or above, regardless of HIV status.
- Microbiological test (molecular or phenotypic) confirming presence of *Mycobacterium tuberculosis*.
- Resistant to at least rifampicin by either molecular or phenotypic drug susceptibility test.
- Completed informed consent form (ICF).

Exclusion criteria:

- Known allergies, hypersensitivity or intolerance to any of the study drugs.
- Pregnant or breast feeding; or unwilling to use appropriate contraceptive measures.
- Liver enzymes >3 times the upper limit of normal.
- Any condition (social or medical) which, in the opinion of the investigator, would make study participation unsafe.
- Taking any medications contraindicated with the medicines in the trial; QTcF >450 ms.
- One or more risk factors for QT prolongation (excluding age and gender) or other uncorrected risk factors for Torsades de Pointes.
- History of cardiac disease, syncopal episodes, symptomatic or asymptomatic arrhythmias (with the exception of sinus arrhythmia).
- Any baseline biochemical laboratory value consistent with grade 4 toxicity.
- Moribund.
- Known resistance to bedaquiline, pretomanid, delamanid or linezolid.
- Prior use of bedaquiline and/or pretomanid and/or linezolid and/or delamanid for one or more months.
- Patients not eligible to start a new course of MDR-TB or extensively drug resistant (XDR)-TB treatment according to local protocol, including but not limited to:
  - Currently on MDR-TB treatment for more than 2 weeks (and not failing).
  - Unstable address.
  - Lost to follow-up in previous treatment with no change in circumstance and motivation.
- Tuberculous meningoencephalitis, brain abscesses, osteomyelitis or arthritis.

The additional criteria for inclusion into the PRACTECAL-PKPD study is for the patient to be aged 18 years or older, to sign the sub-study ICF after agreeing to the additional blood draws.

STUDY OUTLINE

Study period

The first batch of samples' bioanalysis will start in the second quarter of 2021 and continue at regular intervals on batched samples. The data cleaning and analysis will be continuous until end of study.

Study procedures

Patients undergoing recruitment into the TB-PRACTECAL trial will be systematically requested to join the PRACTECAL-PKPD study as well if eligible. After screening and randomisation, only patients who have been randomised to the investigational regimens will be available to be recruited into the PRACTECAL-PKPD.

At least 4 mL (vacutainer tube, lithium heparin) of blood will be collected from the hand, forearm or antecubital vein at each sampling occasion and moment for the PK. The sampling occasions are on Day 1, Weeks 8, 12, 16, 20, 24, 32 and 72 (figure 2). On day 1, blood will be collected just before drugs intake, then 2 and 23 hours after drugs intake. On week 8, blood will be collected just before drugs intake, then 6.5 and 23 hours postdose. These multiple blood sample occasions may require the patient to stay in hospital overnight. At weeks 12, 16, 20 and 24 the blood will be collected just before taking the dose. Both the planned and actual blood collection times should be documented at the earliest opportunity. Samples from week 32 and 72 will be collected whenever feasible after the patients have completed their treatment so blood collection is not relative to drug intake on that occasion. These have been included to capture the elimination phases of the drugs which have long terminal half-lives.

Collected PK blood samples will be centrifuged at 1000 G for 5 min within 30 min of blood drawing, if this is not possible the sample will be refrigerated for a maximum of 60 min. The supernatant plasma will be aspirated and pipetted into two equal aliquots approximately 1.5 mL each and stored in temperature of max −20°C within 60 min of collection, it should then be transferred to dry ice if transport is required and stored in a −80°C freezer. At defined intervals, these frozen samples will be shipped on dry ice to the University of Liverpool laboratory.

Bioanalytical plan

Individual drugs concentrations will be quantified in a Good Clinical Laboratory Practice compliant bioanalytical facility using validated liquid chromatography-tandem mass spectrometry assay methods. Assay performance, sample chromatograms, standard curves and the validity of methods will also be reported.

Visit Number	1	2	3	7	8	9	10	11	12	17
Timing of trial visit	<W -4	D 0	D1	W 8	W 12	W 16	W 20	W 24	W 32	W72
Informed Consent	x	x								
Current medical history and Physical examination	x	x	x	x	x	x	x	x	x	x
PK multiple blood sample			xxx[1]	xxx[2]						
PK trough blood sample[3]					x	x	x	x	x	x[4]
Sputum for culture		x[5]		x	x	x	x	x	x	x
Concomitant medications			x	x	x	x	x	x	x	x
Treatment compliance[6]				x	x	x	x	x	x	x

W=week, D=day

1. Collected in Lithium heparin tubes at 0, 2 and 23 hours post dose
2. Collected in Lithium heparin tubes at 0, 6.5 and 23 hours post dose
3. Collected in Lithium heparin tubes within 30 min pre-dose
4. Where feasible and trial not completed.
5. MIC to B, Pa, Lzd, Cfz and Mfx done
6. Documented timing of first and last drug taken if feasible

Figure 2 The PRACTECAL–PKPD study investigational schedule. Cfz, clofazimine; Mfx, moxifloxacin; PKPD, pharmacokinetic-pharmacodynamic.

Data collection

Demographic data will include age, sex and site. Data for safety outcomes will be collected as part of the main TB-PRACTECAL trial. These include triplicate ECGs at baseline, predose and at 4–6 hours postdose on day 7 and then weekly up to week 8. After week 8, triplicate ECGs predose only every 4 weeks up to week 24, every 8 weeks up to week 48 and then week 72. Full blood count, urea and electrolytes, liver function tests and lipase will be performed on day 0, weekly up to week 8 and monthly up to week 24, at weeks 32, 72 and 108. Audiometry and ophthalmological assessments are also conducted as per investigational schedule. These will be reported as serious adverse events (AE), AE of special interest and other AEs with their respective severity grading using the MSF Severity Grading Scale.[26]

Data for the assessment of efficacy outcomes will be collected as part of the main TB-PRACTECAL trial and include: sputum for smear, culture (time to positivity in MGIT) and MIC at baseline and monthly up to week 24 then every 2 months to week 108. Weight and height at baseline then weight at every visit until completion. Chest X-ray at baseline and week 24.

Other relevant covariate data collected include history of TB treatment and baseline blood glucose levels.

PK-specific data collection

Study-specific electronic clinical report forms will include scheduled sample collection time, actual time sample taken, time separation completed, time stored at −20°C or lower, time last dose taken, prior exposure to drugs of interest, covariates such as time last meal taken, concomitant medications especially ARVs and the time of the last dose.

Data analysis

PK and PKPD models will be developed for each drug based on the plasma concentrations in the study. Nlme modelling software packages (eg, NONMEM or nlmixR) using first-order conditional estimation method with interaction (focei) will be used. Several combinations of absorption models (first order, first order with lag-time and transit absorption), distribution models (one-compartment, two-compartment and three-compartment distribution), variability models (between-subject variability and between occasion variability), and error models (additive, proportional and combined additive and proportional error models) will be assessed. Relative oral bioavailability will be evaluated as a fixed parameter (100% for the population), to allow estimation of the between-subject and between-occasion variability of the relative bioavailability. Competing models will be evaluated during the model building process by the objective function value (OFV—computed as minus twice the log likelihood of the data), physiological plausibility, and goodness-of-fit diagnostics. A significant (p=0.05) improvement will be concluded if the OFV dropped with 3.84 points or more (after the introduction of one new parameter, that is, one degree of freedom). Effects of covariates on PK model parameter estimates

will be assessed using a stepwise covariate modelling approach. During the forward inclusions a p=0.05 will be considered a significant improvement of the model fit while during the backward eliminations a p=*0.01* will be considered significant improvement of the model fit. Model evaluation will include residual plots and visual predictive checks.[27] Prior information in a Bayesian framework will be applied where the venous plasma sampling design fails to support sufficient precision on PK parameter estimates.

Direct linear, E_{MAX} and sigmoid E_{MAX} models will be studied in order to characterise the concentration–effect relationship with a PKPD model. Effects of covariates on PKPD model parameters will be assessed, including the effects of relevant concomitant medications, using identical statistical criteria as described for the PK models in combination with physiological and pharmacological plausibility.

For adverse events both direct and delayed, that is, using an effect compartment, linear, E_{MAX} and sigmoid E_{MAX} models will be investigated to the concentration–effect relationships.

Model development will start as soon as we have the samples from at least 60 patients analysed, that is, interim analysis. Structural models from the interim analysis will be re-evaluated and full covariate analyses will be done once the full dataset becomes available.

For dose optimisations, first, a virtual patient population will be simulated on the basis of observed baseline characteristics among patients enrolled in the study. Then clinically feasible dosing scenarios will be formulated. Lastly, Monte-Carlo simulations done in order to study PK, PKPD and toxicity endpoints following the various dosing scenarios.

REGULATORY AND ETHICAL CONSIDERATIONS

The study has been approved by the MSF Ethics Review Board (reference no. 1541) and the LSHTM Ethics Committee (reference no. 16249) from the two leading institutions. The Belarus RSPCPT ethics committee and the regulator-Centre of Excellence for the Minsk site. PharmaEthics for the Don Mckenzie and Dorris Goodwin hospitals sites, University of Witwatersrand Human Research ethics committee for the Helen Joseph and King DiniZulu Hospitals sites and the South Africa Health Products Regulatory Authority.

The informed consent process will be in line with International Council for Harmonisation of technical requirements for pharmaceuticals for human use (ICH) Good Clinical Practice guidance. The information given and informed consent process will be in the patient's preferred language and documented on a written consent form signed by both the patient and investigator. Where the patient is illiterate, a thumb print of the participant as well as the signature of a witness independent of the study will be documented.

DISSEMINATION

The results of the study will be presented at scientific conferences and published in a peer-reviewed journal. If the TB-PRACTECAL trial successfully identifies effective and safe regimens, the results of this study may be used to inform a WHO guidelines process by potentially answering specific questions on recommended dosages of the B, Pa, Lzd-based regimens as well as potentially informing study countries decisions.

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Additional methodology

Following completion of the data collection, additional clarification in the methodology in achieving objectives 2 and 3 of the PhD was added as detailed in the sections below.

3.4. Data management methods

3.4.1. Study population

Data were obtained from 94 Participants in the TB-PRACTECAL trial (clinicaltrials.gov NCT02589782) recruited from Belarus and South Africa. Participants received one of three investigational regimens. BPaL arm consisted of bedaquiline 400mg daily for 2 weeks then 200mg three times a week for 22 weeks, pretomanid 200mg daily for 24 weeks and tapered dose linezolid 600mg daily for 16 weeks then 300mg for 8 weeks. Clofazimine 100mg daily for 24 weeks was added in BPaLC arm or Moxifloxacin 400mg daily for 24 weeks in BPaLM arm (58). Participants in all three investigational arms contributed samples to linezolid popPK analyses, while participants in BPaLC arm only contributed to clofazimine popPK analyses.

3.4.2. Blood samples

Veinous blood was collected from the participants' ante-cubital fossa on Day 1, Weeks 8, 12, 16, 20, 24, 32 and 72 as detailed in Figure 2 of the protocol publication above.

3.4.3. Covariates

Covariate data were collected as part of the main TB-PRACTECAL trial and included demographic data, HIV status, baseline weight, height, renal function tests, liver function tests and concomitant medications. Creatinine clearance (CLcr), body mass index (BMI) and fat free mass (FFM) were derived covariates.

Creatinine clearance (CLcr) was estimated from serum creatine using the Cockroft Gault equation [3.1]:

Male: CLcr = ((140-age in years)*weight in kg) / (72*creat)

Female: CLcr ((140-age in years)*weight in kg) / (72*creat) *0.85

Body mass index (BMI) was calculated using the formula:

[3.2] BMI = Weight [Kg] /(Height[m])^2

Fat free mass (FFM) was calculated using the formula (59):

[3.3] FFM = $\frac{BMImax*(Ht^2)*Wt}{(Ht^2)*BMImed+Wt}$

Where BMImax is the sex-specific maximum BMI and BMImed is the sex-specific median BMI of the study population, WT and Ht are individual participant's measurements.

3.4.4. Data transformation

A population pharmacokinetic dataset at a minimum consists of the dosing and PK concentration data. However, clinical data is often required for the covariate model. Developing a pop PK dataset is a critical step in the methodology of pop PK modelling and takes the most time (60). It involves collating these different data sources, cleaning and validating the data. Independent validation code in R software was used to develop the pop PK ready datasets and the steps taken are summarised in Figure 3.1 below and detailed in the next sections.

STEP 1: Compile all relevant sources of data

As PRACTECAL-PKPD was a sub-study, the primary source of the demographic, covariate, prescription and treatment adherence data was the TB-PRACTECAL clinical database which was stored in the OpenClinica database. Exports of the relevant clinical research forms (CRF) in csv format were obtained from the DNDi data centre in Nairobi, Kenya. The PRACTECAL-PKPD specific dataset consisted of sample collection, sample processing and sample transport CRFs in a Kobo database. The following data was collected at the trial sites relating to the drug intake:

- Date of visit: DD/MM/YYYY
- PK timepoint: NN
- First dose (ever taken) of drug: DD/MM/YYYY HH:MM

- Last dose (taken) of drug: DD/MM/YYYY HH:MM
- Time started taking IMP: HH:MM
- Time stopped taking IMP: HH:MM
- Dose: NNN mg
- Time sample collected: HH:MM

All doses were directly observed by a health worker in Belarus. In South Africa, only doses taken on day of clinic visit (sample collection day) were directly observed by health workers, home doses were observed by a family member or using asynchronous video directly observed therapy.

The last two CRFs were used for quality assurance, while the first contained data related to the timing of sample collections and drug intake. Each participating site entered the data into electronic CRFs which is merged as one dataset centrally. At time of database development, the Kobo database did not have an audit trail function hence changes following data queries were recorded in a separate file. The frozen plasma samples were shipped to The University of Liverpool Bioanalytical Facility, the bioanalysis results were obtained at two different occasions exported as csv files.

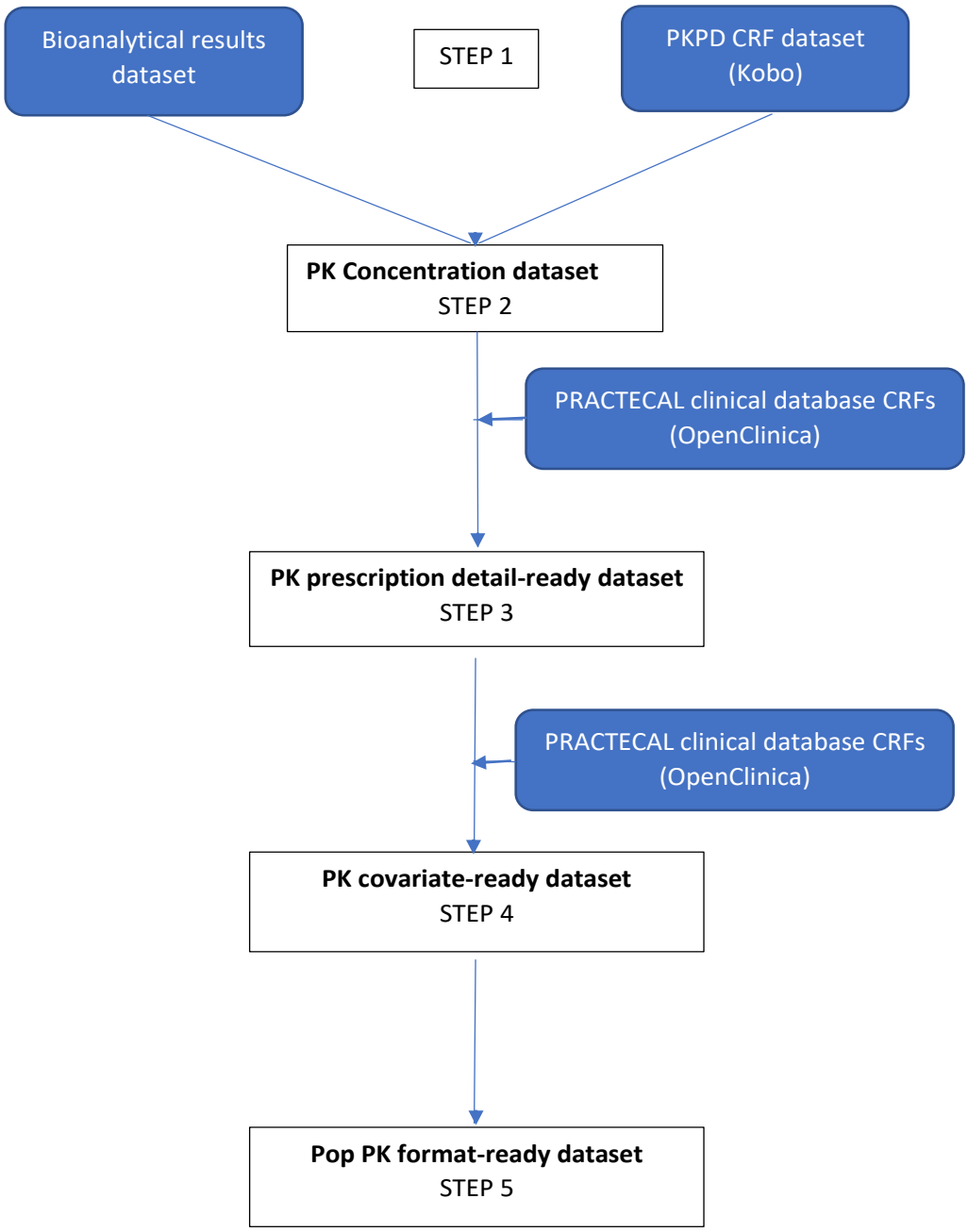


Figure 3.1: Infographic of summary of the data transformation process. Blue boxes are the source databases and white boxes are the compiled datasets.

STEP 2: Drug concentrations data cleaning and validation

The data cleaning and validation was conducted in multiple steps. Where missing data was identified, queries were sent to research sites or the bioanalytical facility and often these were resolved. All PK concentration results reported as being below the lower limit of quantification (<LLQ) were handled as missing, but for two different reasons. Firstly, the scenario where the drug was never expected to be in the patients' blood. This applied if there was no record of a drug being prescribed nor taken before the patient joined the clinical trial or when at least eight weeks had passed since the last dose (trial visit weeks 32 and 72) for pretomanid and linezolid. Secondly, during treatment when the drug could have been present in the blood. Since the second scenario <LLQ results constituted less than 20% of all sampling timepoints, the data was censored and the sample results coded as missing (61). We used the remaining values as if they came from a full distribution as in the so-called M1 method (62).

STEP 3: prescription data cleaning and validation

Validation and cleaning of prescription data involved establishing which drug, at what dose, was taken at what time relative to the blood sample collections. Where there was a discrepancy between the Kobo and Openclinica data, the Openclinica data were used because they had undergone source data verification during trial implementation.

STEP 4: Covariate data cleaning and validation

Validation and cleaning of covariate data involved selecting the relevant CRFs from the clinical database and merging them with the prescription-ready dataset. There were no missing covariate data. The choice of effect covariates to be included in the dataset were based on known metabolic and elimination pathways of the drugs (63, 64), previously published covariates and limited to those that were collected and reported in the main trial (58). The following covariates were explored for all drugs:

- Age, sex, weight, height, race and (derived) the fat free mass and BMI
- HIV infection status
- Renal function tests: BUN, creatinine and creatinine clearance (Cockroft Gault equation)

- Liver function tests: Alkaline phosphatase (ALP), Bilirubin, Alanine transferase (ALT), Aspartate transferase (AST), total protein (TP), Albumin
- Treatment regimen

STEP 5: Pop PK format data set generation

Generation of a poppk format data was carried out to meet the requirements of the nonlinear mixed effects modelling software (nlmixr2). Each line of data needed to have the following fields:

- Subject identification (ID)
- Identification of the timepoint of visit number (VISIT),
- Identification of the timepoint of sample collection (PTIME)
- Date and actual time of sample collection (PDATE)
- The date and time of first dose (START)
- Time after first dose or observation (TIME)
- Measured drug concentration or dependent variable (DV)
- Missing data value (MDV)
- Dosing record (AMT)
- Compartment code for observation/dosing record (CMT)
- Inter-dose interval (II)
- Additional identical dose given (ADDL) derived from first dose and last dose timing.

A sample of the data set for one patient on two visits is shown below.

ID	VISIT	START	LD	PDATE	TIME	DV	MDV	EVID	CMT	AMT	ADDL	II	PTIME
1	3	25/09/2019 09:50	25/09/2019 09:50	NA	0	0	1	1	depot	200000	54	24	0
1	3	25/09/2019 09:50	NA	25/09/2019 09:20	0	0	1	0	centr	0	0	0	0
1	3	25/09/2019 09:50	NA	25/09/2019 11:50	2	8512.06	0	0	centr	0	0	0	2
1	3	25/09/2019 09:50	NA	26/09/2019 08:50	23	0	1	0	centr	0	0	0	23
1	7	25/09/2019 09:50	19/11/2019 10:04	NA	1321.2	0	1	1	depot	200000	0	0	0
1	7	25/09/2019 09:50	NA	20/11/2019 08:45	1343.9	304.31	0	0	centr	0	0	0	0

The measured drug concentrations (DV) for each subject were then plotted against the date of sample collection (PTIME) in an overlapping plot of the dosing record (AMT) in order to identify any outliers.

3.5. Bioanalytical methods

The analytes were extracted from plasma using protein precipitation with 80:20 (v/v) methanol: acetonitrile. Quantification was performed using reverse phase high performance liquid chromatography (HPLC) interfaced with a triple quadrupole AB Sciex 6500 mass spectrometer, operating in positive ionisation mode. Stable isotopically labelled internal standards, bedaquiline-d6, clofazimine-d7 and linezolid-d3 were included in the sample extraction procedure to correct for any variation in extraction efficiency and ion suppression effects.

Assay validation was performed prior to the analysis of clinical samples, and in accordance with FDA and EMA guidelines. The lower limit of quantification (LLQ) is defined as the lowest concentration for which the percentage deviation from the nominal standard concentration is less than 20%. For all other calibrators and QC samples, mean concentrations should be within ±15% of their nominal level and the %CV should not exceed 15%. The LLQ for bedaquiline, pretomanid, linezolid and clofazimine were 20 ng/mL, 7 ng/mL, 80ng/mL and 7ng/mL respectively.

Data acquisition and integration was performed by Analyst version 1.6.1 and Multi Quant version 3.0, respectively.

3.6. Population Pharmacokinetic model building methods

A time series data analysis was conducted with nonlinear mixed effects modelling. Fixed effects determined by the structural model describe the variability of the parameter estimates in the population. Random effects are determined by the statistical model and describe the 'unexplainable' variability of parameter estimates across individuals such as between occasion variability. The covariate model describes the variability that 'can be explained' or predicted by differences in individual characteristics such as age. A population pharmacokinetic model building process aims to identify the model that optimises the three model components (see figure 3.2 below).

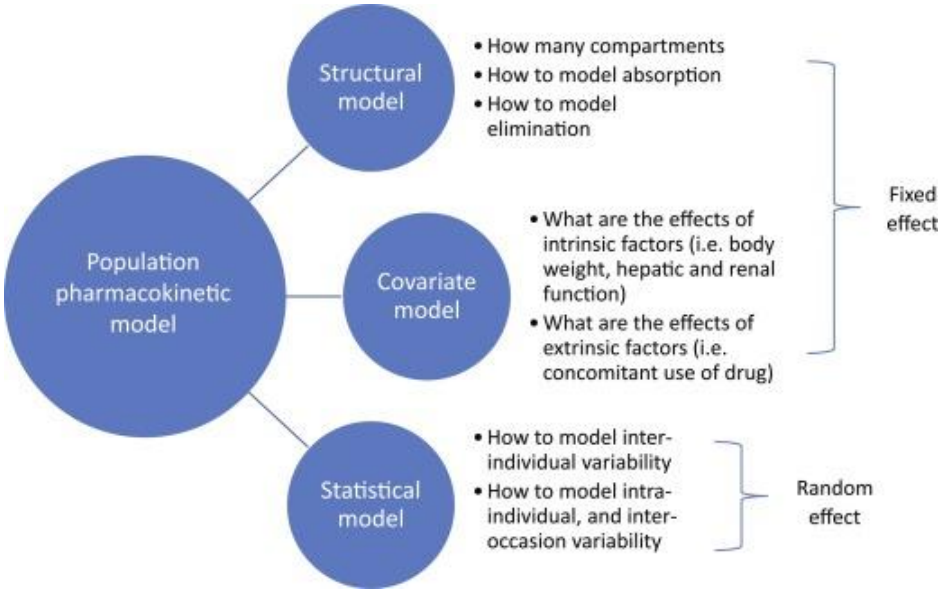


Figure 3.2: Key components of a population pharmacokinetic model(65)

3.6.1. Software

All analyses were conducted using R software version 4.2.2 (66), nlmixr2 package (67) and the RStudio interface. Both first-order conditional estimation method with interaction (FOCEI) and stochastic approximation expectation-maximisation (SAEM) algorithms were used (68, 69) as they have different advantages and disadvantages including speed, robustness to initial parameter estimates, and stability in overparameterized models and parameter precision (70).

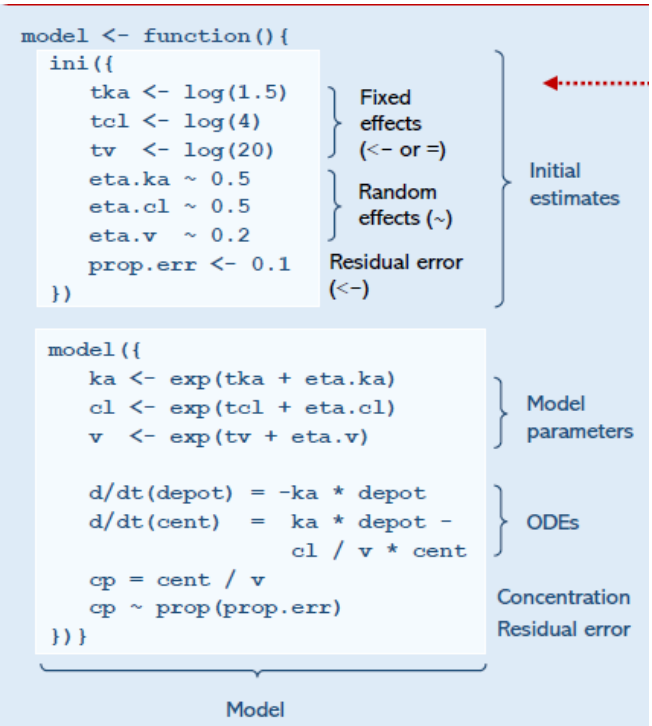


Figure 3.3 example of model code (adapted from "nlmixr Cheat Sheet")

3.6.2. Base Structural model

The base model was selected using the following criteria/assessments:

- Expected model based on individual concentration plots
- Objective function value (OFV),
- Standard error of parameter estimates
- Between subject variability (BSV)
- Goodness of Fit (GoF) plots
- ETA and EPS shrinkage

3.6.3. Structural model

One, two and three compartments distribution models with linear elimination were tested. Based on previously published popPK models, absorption model options explored included transit absorption, lag time and fixed absorption rate constant (ka) models.

3.6.4. Statistical model

Residual variability (intra and inter-individual) variability was explored in the PopPK analyses. Residual error models with additive, proportional and combined additive and proportional components were tested.

Equation [3.4]

$$Y_{ij} = F_{ij} + \varepsilon_1$$

$$Y_{ij} = F_{ij} * (1 + \varepsilon_{1ij})$$

$$Y_{ij} = F_{ij} * (1 + \varepsilon_1) + \varepsilon_{2ij}$$

Where Y_i is the j^{th} observed concentration for the i^{th} subject, F_{ij} is the corresponding model predicted concentration for subject i, ε_{1ij} and ε_{2ij} are two independently normally distributed residual error variables (mean = 0, sd = σ_{2i} and mean = 0, sd = σ_{2i}).

The inter-individual random effects on the parameters were modelled using exponential model assuming a lognormal distribution described by:

[3.5] $P_{in} = P_n * e^{\eta in}$

Where P_{in} is the n^{th} PK parameter value for i^{th} individual, P_n is the n^{th} typical parameter value for the population, η_{in} is normally distributed random effect (mean = 0, sd = ω_{2n}).

3.6.5. Covariate model

The covariate relationships were screened by plotting the base model's Empirical Bayes Estimates of the parameters (e.g. clearance and volume of distribution) against the potential covariates. Further exploration was done using the etas against the potential covariates. The individual covariates were also tested for collinearity. The power model used for describing continuous covariate impact was:

[3.6] $$\theta_i = \theta_{Param} * \left(\frac{COV}{MEDIAN}\right)^{Power}$$

Where θ_i is the individual PK parameter value, θ_{Param} is a typical PK parameter value.

For categorical covariates, such as sex, the effect was modelled as follows (Eq. 9):

$$[3.7]\ \theta_i = \theta_{TV} \times (1 + cov_i \times \theta_{cov})$$

Where cov_i is a dummy variable that took on a value of 1 or 0.

The chosen covariates were added in a stepwise fashion using a forward inclusion approach, with the most significant covariate being entered first; a reduction in the objective function value (OFV) of 3.84 at a p value of <0.05 was considered statistically significant. Backward elimination was also performed for covariates that yielded a drop in OFV of 6.63 at a p value of <0.01 or 10.83 at a p value of <0.001 when other covariates were added.

3.6.6. PopPK model evaluation

The final identified model (s) underwent the follows evaluations:

- Assessment of goodness-of-fit (GOF) plots,
- Visual predictive checks
- Plausibility of parameter estimates and their precision,

Non-parametric bootstrap using 1,000 simulations were done for linezolid, clofazimine and bedaquiline and reported the median result and the 95% confidence interval (CI).

3.7. Pharmacokinetic and pharmacodynamic analyses

3.7.1. Pharmacokinetic-pharmacodynamic indices

Pharmacokinetic-pharmacodynamic indices such as the concentration-dependent index, area under the concentration-time curve from zero to twenty four hours ($AUC_{0\text{-}24}$) divided by the minimum inhibitory concentration (MIC) and time-dependent index, percentage of the dosing interval during which the plasma concentration exceeds the MIC (%T>MIC) are used to indicate the relationship between drug exposure and a microbiological measure of susceptibility in antimicrobials.

3.7.2. Pharmacokinetic-pharmacodynamic targets

PKPD targets of previously published AUC/MIC and T%>MIC indices for the study drugs were used as predictors of efficacy. AUC/MIC PKPD targets were used for bedaquiline,

pretomanid, linezolid, and clofazimine while the %T>MIC targets were additionally used for pretomanid and clofazimine. Targets ranged from those for net static effect (bacteriostatic), 1 log kill and minimisation for the selection of resistance depending on the availability of previously published targets. These targets were developed using BALB/c mice infection models (pretomanid, clofazimine) or hollow fibre system for TB (linezolid and bedaquiline).

3.7.3. Determination of minimum inhibitory concentration

Baseline MICs were available for isolates from 465, 478, ,406 and 464 patients in the TB-PRACTECAL trial for linezolid, pretomanid, clofazimine and bedaquiline. The MIC testing was performed using BACTEC MGIT 960 instrument on pre-treatment isolates after confirming presence of M. tuberculosis complex. The concentrations tested for these drugs are 2-fold serial dilutions across the following ranges:

- Bedaquiline: between 8 and 0.0016 mg/L
- Pretomanid: between 8 and 0.016 mg/L
- Linezolid: between 1 and <0.063 mg/L
- Clofazimine: between 2 and 0.32 mg/L

3.7.4. Probability of target attainment (PTA)

The final popPK model for each drug was used to construct simulated PK profiles for individual patients at various trial and hypothetical doses and assumed protein binding levels using the Monte Carlo simulation. 1,000 stochastic simulations from the study population resulting in a range between 2,000 and 3,000 virtual patients were performed. Number of patients attaining the various AUC/MIC and T%>MIC efficacy targets at the observed range of MICs of patients in the TB-PRACTECAL trial and at the WHO defined critical concentrations or clinical break points (71) were simulated.

Chapter 4: Linezolid population pharmacokinetics and probability of pharmacodynamic target attainment in participants in the TB-PRACTECAL clinical trial

4.1. Introduction

This chapter summarises the methods for the study and detail out the results of the population pharmacokinetics of linezolid and probability target attainment. The results section describes the participants of the study, the pharmacokinetic data that was used and the linezolid population pharmacokinetic model building. The intermediate model building steps describe the structural, statistical and covariate model building and evaluation of the final linezolid population pharmacokinetic model. The primary parameters and secondary parameters' empiric bayes estimates derived from the final model are presented, including a discussion on how they compare with those from previously published papers. The linezolid MICs in the parent PRACTECAL study are presented and used in discussing the probability target attainment analyses. These results are then discussed considering the results of the TB-PRACTECAL trial (72) and other published evidence on the use of linezolid in treatment of tuberculosis.

4.2. Methods

4.2.1. Study design

This was a sub study nested in the TB-PRACTECAL randomised controlled trial in patients with rifampicin resistant tuberculosis. Participants received one of three investigational regimens. BPaL arm consisted of bedaquiline 400mg daily for 2 weeks then 200mg three times a week for 22 weeks, pretomanid 200mg daily for 24 weeks and tapered dose linezolid 600mg daily for 16 weeks then 300mg for 8 weeks. Clofazimine 100mg daily for 24 weeks was added in BPaLC arm or Moxifloxacin 400mg daily for 24 weeks in BPaLM arm. Blood samples were collected on Day 1 (0, 2 and 23 hours), Weeks 8 (predose, 6.5

and 23 hours), 12, 16, 20, 24, 32 and 72 post randomisation visits. Drug concentrations were quantified in a GCP laboratory using a high-performance liquid chromatography-tandem mass spectrometry. The lower limit of quantification for linezolid was 80ng/mL.

4.2.2. Pharmacometric analysis

nlmixr2, an open-source R package was used for population PK modelling and simulation estimation. R v4.4.1 was used for dataset creation, data exploration and generation of tables and plots. The list of r packages used is in appendix 7. The PopPK for linezolid was analysed using a non-linear mixed effect modelling approach. The first-order conditional estimation with interaction (FOCE-I) algorithm in nlmixr2 was used. Inter-individual variability (IIV) at the parameter level and residual variability (RV) at the observation level made up the mixed effects analysis.

4.2.3. Structural model

The PopPK study first explored basic model structure based on the observed plasma concentration data. One and two-compartment linear models were evaluated respectively with combined, proportional and additive residual error models. Finally, random effects on clearance (CL) and volume of distribution (V) without correlation were included in the model. A log-transformed residual error model was also tested. Various absorption models were explored including transit compartment models and fixed absorption constant (ka).

4.2.4. Covariate model

A covariate matrix of age, sex, weight, BMI, FFM, race, BUN, ALT, AST, TP, CLCR, treatment regimen and eta estimates on clearance and volume of distribution from the base model explored correlation as well as covariate collinearity. FFM allometric scaling was applied to both volume of distribution and clearance. The coefficients of the power model were fixed to 1 for V and 0.75 for CL. The selected covariates underwent stepwise forward inclusion ($P<0.05$, $\Delta OFV > 3.84$) and backward elimination ($p<0.001$, $\Delta OFV > 10.83$) to select those that would improve the model fit significantly.

4.2.5. Model evaluation

Goodness-of-fit plots were used to assess how well the model predicted individual and population values closely matched the observed PK data. Model validation was also performed using visual predictive check (VPC) plots and bootstrapping (73, 74). The shrinkage, relative standard error, and variability value including omega and sigma values were also used to assess the precision and robustness of the model.

4.2.6. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations were determined from a routine testing concentration set (1, 0.5, 0.25 mg/L) in MGIT; testing was performed using a higher (32, 16, 8, 4, 2 mg/L) or lower (0.125, 0.016 mg/L) testing concentration set if required. The results from all participants from the TB-PRACTECAL trial were summarised by country of enrolment and the median and interquartile range were reported.

4.2.7. Probability of Target Attainment

Recent publications have tended to use the linezolid efficacy target in treating tuberculosis in adults as an *f*AUC$_{0\text{-}24}$/MIC ratio of 119 (75, 76) or as high as 125 (77). The 119 target was first published by Srivastava *et al*. based on hollow fibre system experiments and should more accurately be described as the exposure associated with 80% of maximal kill (EC$_{80}$) *f*AUC$_{0\text{–}24}$/MIC ratio at day 28 with linezolid monotherapy. In the study, an *f*AUC$_{0\text{-}24}$/MIC ratio of 16.24 was the exposure associated with bacteriostasis, while 73.60 was associated with 1.0 log10 kill. The estimated time to negative culture was 68 days in the 73.60 *f*AUC$_{0\text{-}24}$/MIC ratio and 46 days for 111.20 and 45 days for 157.30 *f*AUC$_{0\text{-}24}$/MIC ratios. *f*AUC$_{0\text{-}24}$/MIC ratio exposures of 111.20, 157.33 and 73.60 completely sterilised the media on study day 35 while a ratio of 43.47 was the lowest exposure enabling sterilisation and this occurred on study day 42 (78). Protein bound proportion was estimated at 31% (51).

We explored the probability of attaining the various efficacy targets in first 16 weeks where the dose was given at 600mg and in the latter 8 weeks when it was given at 300mg

daily. The PK/PD breakpoint was defined as the highest MIC at which the probability of target attainment is >90%.

4.3. Results

4.3.1. Study population

94 participants in the TB-PRACTECAL trial (clinicaltrials.gov NCT02589782)(72) who were randomised to receive one of three investigational regimens taking linezolid in the PKPD sub study (clinicaltrials.gov NCT04081077) (79) were included in the study. 34 (36%) of the participants were female, they had a median age of 36 years (range: 19 – 71 years) see table 4.1 and they contributed 952 timed plasma samples which upon bioanalysis were included in the linezolid PopPK dataset. 297 (31%) of the observations were below the limit of quantification, 74 of these were collected before the first dose (day 0) and 133 were collected more than four weeks after the last dose (study weeks 32 and 72). 90 (14%) of the 655 recorded plasma concentration measurements were below the limit of quantification during treatment.

Observed linezolid concentration ranged from 46.78ng/ml to 30,650.15 ng/ml. The median trough concentration was 619.58 ng/ml (mean was 3503.83 ng/ml), with an interquartile range of 232.71 to 1556.51 and shown in figure 4.1. Individual drug profiles are included in the appendix 6.

Table 4.1: baseline characteristics of study participants (n = 94)

Characteristic	Total
Female, n (%)	34 (36)
Age, median years (range)	36 (19-71)
Race, n (%)	
Asian	1 (1.1)
Black	50 (63.7)
Caucasian	39 (42.9)
Other	1 (1.1)
HIV status, n (%)	
positive	36 (39.6)
negative	54 (59.3)
not known	1 (1.1)
Regimen, n (%)	
BPaLM	38 (40)
BPaLC	30 (32)
BPaL	26 (28)
Weight, kg	56.8 (39.2 – 144.4)
Height, cm	170 (145 – 196)
BMI, Kg/m2	19.7 (13.3 – 47.2)
Fat Free Mass, kg	45.5 (28.6 – 75.5)
BUN (mmol/L)	3.6 (1.7 – 8.5)
ALT (IU/L)	19.5 (4 – 113)
AST (IU/L)	22 (4 – 82)
ALP (IU/L)	67 (37 – 132)
Albumin* (g/L)	44 (36 – 49)
Total protein* (g/L)	77 (61 – 118)
Creatinine (mcrmol/L)	66 (35 – 111)
Creatinine clearance (mL/min)	105.4 (43.4 – 243.8)

Median (min-max) if not stated otherwise. * n=39

BPaLM = bedaquiline+pretomanid+linezolid+moxifloxacin, C=clofazimine,

BMI= body mass index, ALT= alanine transaminase, AST= aspartate aminotransferase, ALP=alkaline phosphatase, BUN= blood urea nitrogen

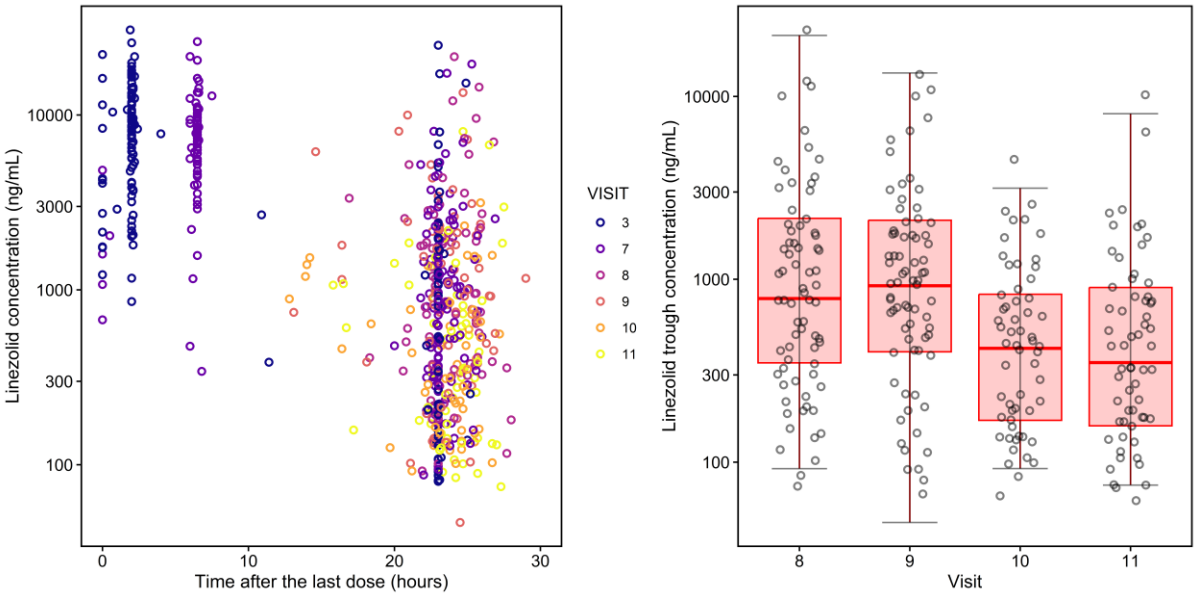


Figure 4.1: Plots of observed linezolid concentrations by time after last dose on the left. Trough (visits 8-11 [weeks 12, 16, 20, 24]) concentrations by time after first dose aggregated by study visit number (right). The pink box represents the interquartile range with the red horizontal line representing the median, while the whiskers represent the 5th and 95th percentiles.

4.3.2. Structural and variability model

One and two compartments distribution models were explored using both FOCEI and SAEM algorithms. The linCmt, a pseudo-function in nlmixr2 which figures out the type of model to use based on the parameter names specified was also explored. However, as some one-compartment models couldn't converge with the linCmt function, all subsequent models used ordinary differential equation (ODE) function. Focei algorithm performed better and ran faster than Saem so all subsequent model comparisons were run using focei. The summary of model evaluation results with type of algorithm, OFVs and delta ΔOFVs is in supplementary appendix S1.

Transit compartment models did not converge and given the limited number of samples taken during the absorption phase (only day one and week eight occasions had samples collected before 8 hours after last dose) in the study, further exploration of absorption models was deemed not beneficial. A fixed absorption rate constant from a previously published model (80) was used.

A both sided log-transformation, with an additive error on log-transformed data (OFV 9729.79), proportional (OFV 9981.71) and combined additive and proportional (OFV 9980.43) residual error models were tested for the one compartment structural model. The additive error model on log transformed data also performed best amongst the two compartment models (see table in appendix S1).

The absorption rate constant (ka), clearance (cl) and volume of distribution parameters of the four models with the lowest OFVs were estimated and compared, presented in appendix S2. Shrinkage on clearance was found to be within an acceptable range for all four models (81). The estimated clearances were comparable to values that have been previously published while the volume of distribution was within the expected range for the one compartment fixed Ka model, another had very low volume (~9L) and the two compartment models with the best OFVs had volumes that were too high (~100L).

The selected base model was therefore a one compartment disposition model with first-order absorption and elimination, and a fixed absorption represented by figure 4.2 and the differential equations below. An exponential model was used to estimate the residual error. The goodness of fit (GOF) plot showed that the base model accurately fit the data and the VPC plots confirmed the base model's predictions to adequately reflect the observed concentration data.

Equation [4.1]

$$\frac{dA_{depot}}{dt} = -K_a \times A_{depot}(t)$$

$$\frac{dA_{central}}{dt} = K_a \times A_{depot} - \frac{CL/F}{V_c/F} \times A_{central}(t)$$

where A_{depot} is the amount of linezolid in the depot compartment. where $A_{central}$ is the amount of linezolid in the central compartment. K_a is the absorption rate constant for the transfer of linezolid from depot compartment to central compartment. CL/F is the apparent clearance of linezolid. V_c/F is the apparent volume of distribution of linezolid.

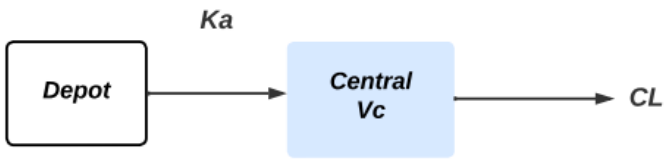


Figure 4.2: Schematic representation of the structural linezolid model.
K_a = absorption rate constant, V_c = central compartment, CL = clearance

4.3.3. Covariate model

On covariate visual exploration, potential linear relationships were noted on parts of the Loes line on age, weight, time varying weight and body surface area on both etas on clearance and etas on volume of distribution. Race also appeared to impact both eta clearance and eta volume of distribution, plots are in appendix 2. The only observed collinearity was in body size covariates (see figure S2.4).

31% of linezolid in plasma is protein bound, it undergoes hepatic metabolism and up to 30% of unchanged drug is excreted in urine (82), therefore covariates measuring serum protein (Total protein - TP), renal function (Blood Urea Nitrogen (BUN), Creatinine clearance (Cr_{Cl}) and liver function (ALT, AST, Total bilirubin) were planned for further exploration in the models. Age, weight, height, body max index (BMI), fat-free mass (FFM), Caucasian race and black race were chosen based on the visual exploration. Sex, HIV infection status and treatment arms 1, 2 and 3 were also included as they were explored in previously published studies.

Allometric fat free mass and Caucasian race on clearance were the retained covariates (ΔOFV -20.5 from selected base model) in the backward step at $p<0.001$. Age (ΔOFV -4.59) and creatinine (ΔOFV -3.93) clearance on clearance were identified in the first cycle, while black race on both clearance (ΔOFV -11.53) and volume (ΔOFV -11.95) and Caucasian race on volume (ΔOFV -16.27) were identified in the second cycle (see detailed results and steps in appendix 3). The final model code is shown in appendix 8.

4.3.4. Final model evaluation

Goodness-of-fit plots for the final PopPK model showed no significant bias from the unity line in both PRED VS DV and IPRED VS DV, indicating that the model predicted individual and population values closely matched the observed PK data (Figure 4.3).

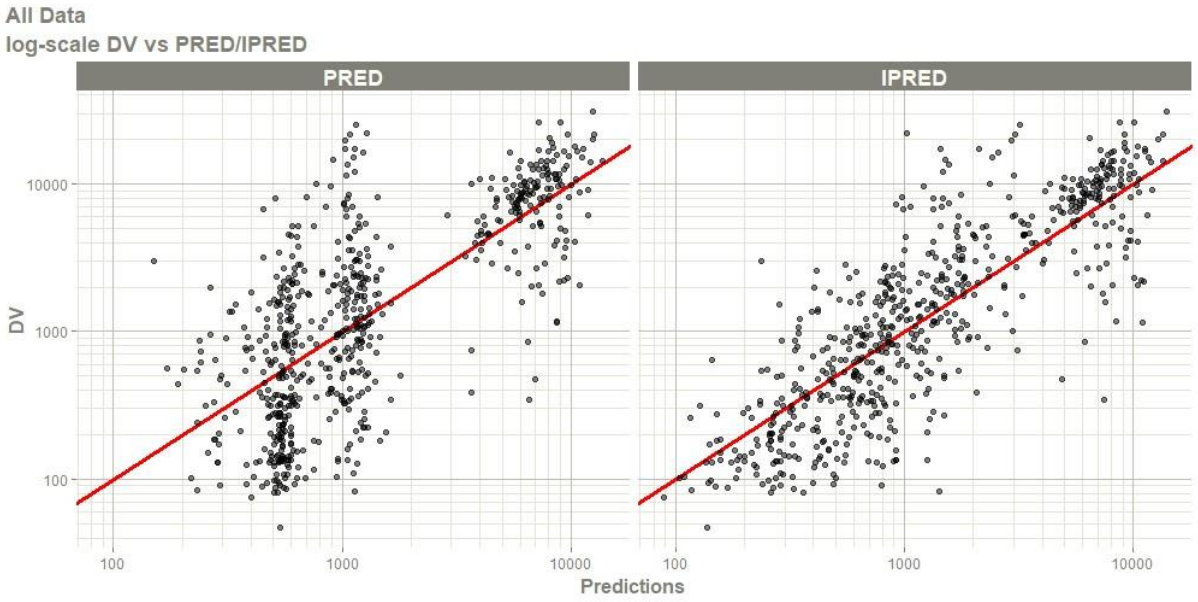


Figure 4.3: Final linezolid model goodness of fit plot of observed data plotted against predicted data (DV vs. PRED) on a log scale

In both CWRES vs population prediction (figure 4.4) and CWRES vs time (figure 4.5), all the CWRES data points were within +/- 3 and fairly evenly distributed, suggesting no significant systematic deviations in model fit.

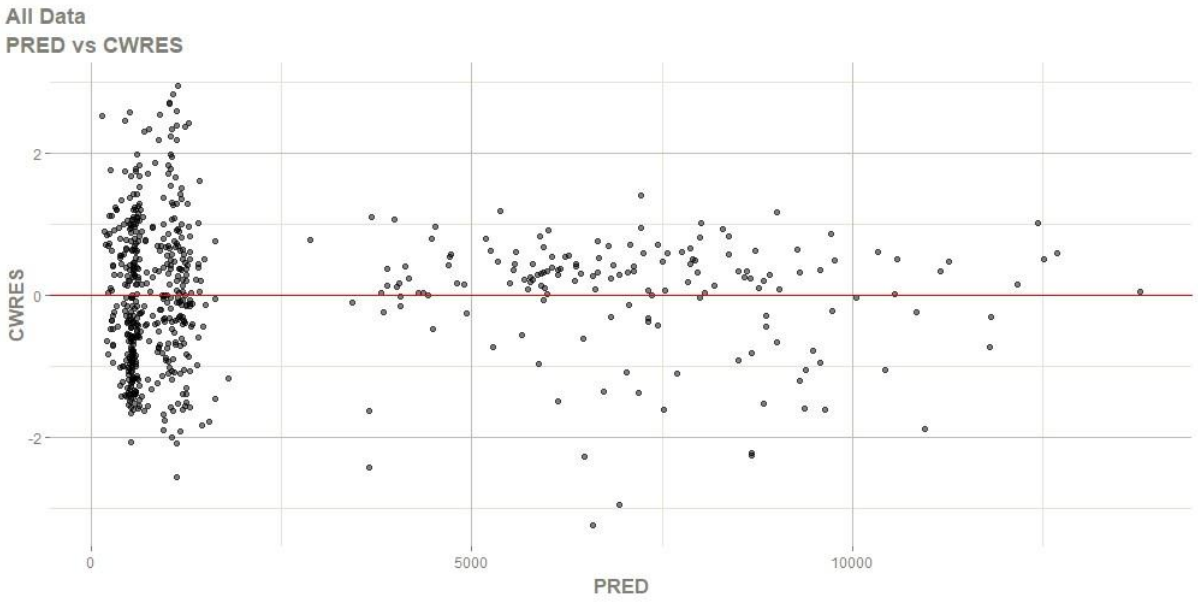


Figure 4.4: Final model goodness of fit plot of the conditional weighted residuals versus the predicted data (PRED vs CWRES)

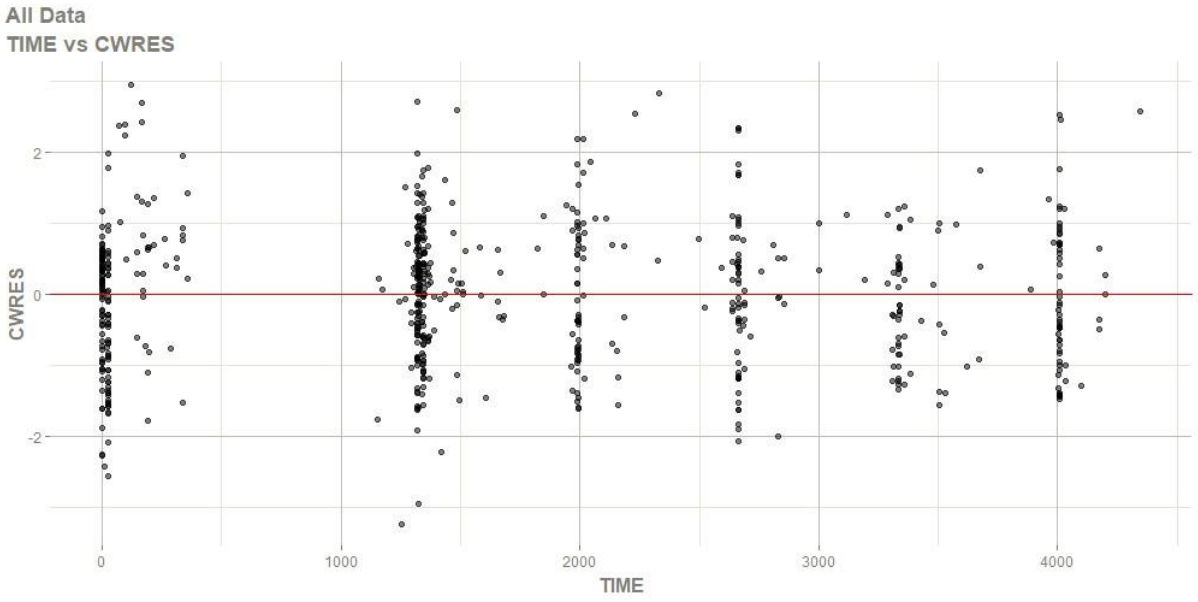


Figure 4.5: Final model goodness of fit plot of the conditional weighted residuals versus time after first dose

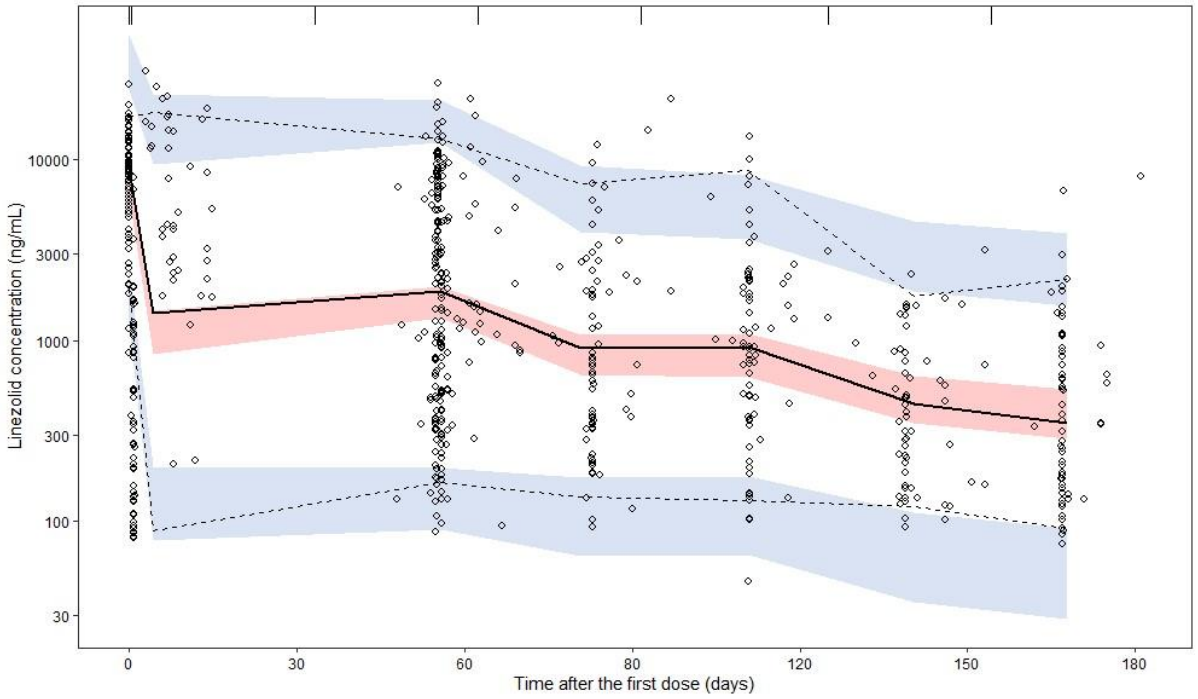


Figure 4.6: Linezolid final model visual predictive check plotting concentrations against time after first dose. The black circles in the figure represents the observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. The purple area represents a simulation-based 95% confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink.

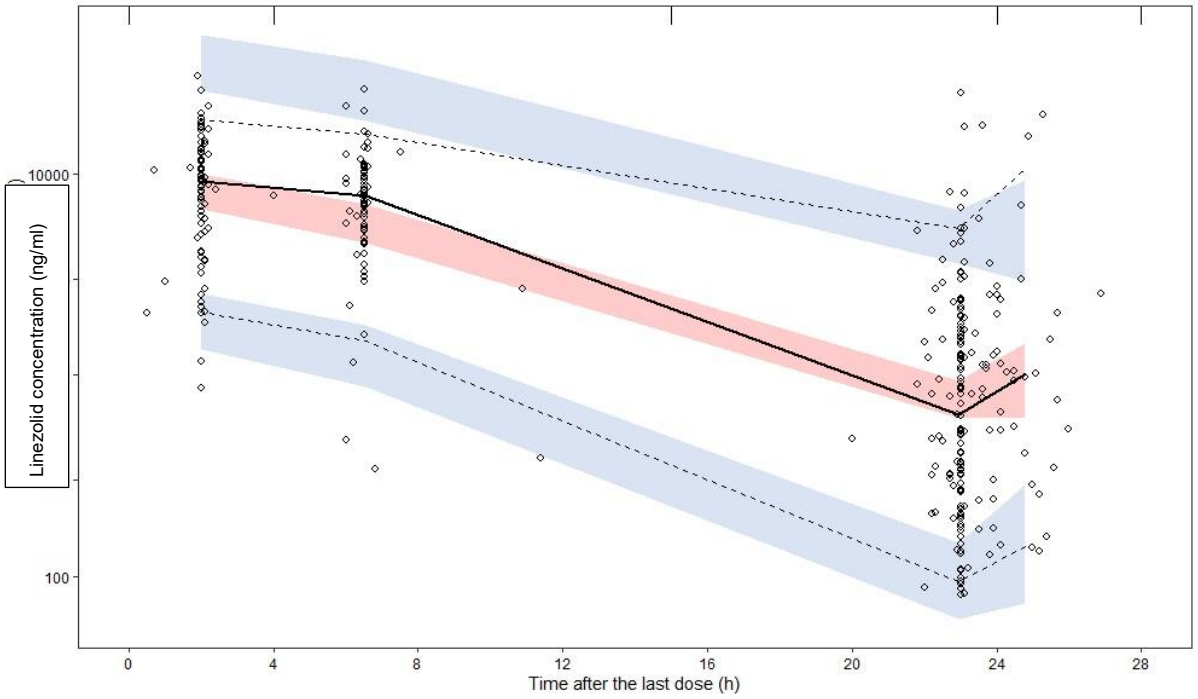


Figure 4.7: Linezolid final model visual predictive check of 24-hour profile, plotting concentrations against time after last dose. The black circles in the figure represents the

observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. The purple area represents a simulation-based 95 % confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink.

In the time after first dose VPC (figure 4.6), almost all observed values at 5th, 50th and 95th percentiles were within the 90% CI of predicted values, the time after last dose VPC (figure 4.7) had minor overestimation in the 95th centile in the first 8 hours. Overall demonstrating the predictive accuracy of the final model.

Covariate matrix of empiric bayes estimates of clearance and volume of distribution in the final model demonstrated a limited variability by body size covariates on clearance, and negligible variability by all other explored continuous covariates (supplementary appendix S4.1). Treatment regimen, sex and HIV status categorical covariates did not show any observed differences in both apparent clearance and volume of distribution. However, there was a tendency of higher clearance in Caucasian and lower clearance in black race study participants, reconfirming race as the only covariate in addition to FFM allometry that was influential in the final model. (supplementary appendix figure S4.2).

4.3.5. Final model parameter estimates and bootstrap

The reliability and stability of this final model were verified using 1000 bootstrap samples. The median values of estimated parameters obtained using bootstrap analysis (table 4.2) were consistent with corresponding values within the final model, thus reflecting the final model's stability.

Table 4.2: Estimated linezolid population pharmacokinetic parameters in base and final models

Parameter	Parameter estimate (RSE[%])		Bootstrap Median (95% CI)
	Base model	Final model	
OBJ	9716.564	9696.057	
-2LL	-5441.808	-5431.555	
Fixed-effect parameters			
Ka, h-1	1.23 (fixed)	1.23(fixed)	1.23 (fixed)
CL, L/hr	6.59 (2.8)	5.88 (3.1)	5.88 (4.96 – 6.96)
θcl, FFM	0.75	0.75	0.75
θcl, caucasian		0.276 (0.155, 0.397)	0.276 (0.16 – 0.392)
V, L	58.4 (1.53)	58.5 (1.58)	58.5 (49.7 – 68.8)
Random-effect parameters			
η(cl), %	29.9	25.0	26.0
η(v), %	5.66	5.66	5.66
Residual error parameters			
ε	0.89	0.888	0.888

OBJ = objective function value , -2LL = 2 x the log likelihood , θcl, FFM = FFM theta on clearance , θcl, Caucasian = caucasian theta on clearance , η(cl) = eta on clearance , η(cl) = eta on volume of distribution, ε = epsilon = intraindividual variability/RUV

Linezolid exposure parameters were estimated from the final model's empiric Bayesian estimates and presented in table 4.3.

Table 4.3: Final linezolid secondary pharmacokinetic parameters

Parameter (unit)	Median	Interquartile range	range
Day 1 600mg dose			
Tmax (hr)	2.0	2.0 - 2.3	1.8- 2.5
AUC 0-24 (mg hr/L)	85.47	70.23 - 102.06	35.43 - 152.57
AUC 0-∞ (mg hr/L)	92.27	73.62 - 116.32	36.12 - 179.44
Ctrough (mg/L)	0.74	0.48 - 1.21	0.12 - 2.54
Cmax (mg/L)	8.08	6.95 - 9.45	4.49 - 12.88
Steady state 600mg dose			
Tmax (hr)	2.0	2.0 - 2.3	1.8- 2.5
AUC 0-24 (mg hr/L)	90.40	66.40 - 110.44	35.45 - 185.40
AUC 0-∞ (mg hr/L)	92.03	67.44 - 116.36	36.12 - 179.44
Ctrough (mg/L)	0.71	0.41 - 1.22	0.12 - 3.18
Cmax (mg/L)	8.80	6.92 - 10.41	4.50 - 14.99
Steady state 300mg dose			
Tmax (hr)	2.0	2.0 - 2.3	1.8 - 2.3
AUC 0-24 (mg hr/L)	45.67	35.46 - 57.87	18.07 - 90.17
AUC 0-∞ (mg hr/L)	45.66	34.17 - 57.92	18.06 - 89.72
Ctrough (mg/L)	0.39	0.24 - 0.69	0.06 - 1.58
Cmax (mg/L)	4.49	3.51 - 5.28	2.29 - 7.27

4.3.6. Final model's plausibility of parameter estimates

To review linezolid parameter estimates from population pharmacokinetic models, we searched the PubMed database from inception to February 2024 using the following search terms: "(linezolid) AND (population pharmacokinetics) AND (drug resistant tuberculosis)". The search identified 21 unique publications; after excluding reviews, in vitro and paediatric studies, 9 publications (see table 4.4) reporting primary population pharmacokinetic parameters in adults were retained (75, 80, 83-89). Reported linezolid

clearance ranged from 3.57 to 7.69 L/hr while apparent volume of distribution ranged from 31.2 to 50.7 L.

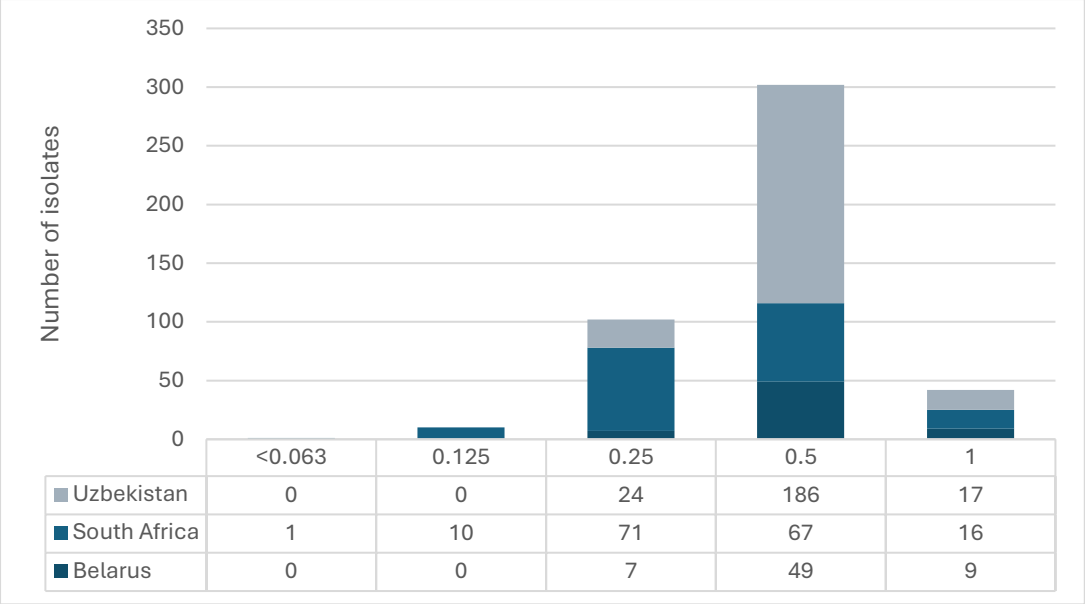
Table 4.4: Previously published linezolid population pharmacokinetic models and the estimated primary parameters

Publication	Year of publication	Ka (1/l)	clearance (l/hr)	volume (l)
Resendiz-Galvan JE *et al.*	2023	2.31	3.81	31.2
Mockeliunas L *et al.*	2022	1.8	6.3	50.6
Zhou W *et al*	2022	1.23 (fixed)	4.59	44.5
Tietjen AK *et al.*	2021	0.679	7.69	45.2
Abdelwahab MT *et al.*	2021	1.22	3.57	40.2
Alghamdi WA *et al.*	2020	1.65	6.32	40.6
Strydom N *et al.*	2019	2.13	5.94	50.7
Kamp J *et al.*	2017	1.021	5.39	0.661 (l/kg)
Alffenaar JW *et al.*	2010	0.939	6.1	0.654 (l/kg)

The estimated clearance of 5.88 L/hr in this study is within the range of previously published reports and the volume of distribution is the highest reported but close to the higher ones (77, 80).

4.3.7. Minimum inhibitory concentrations (MIC)

The distribution of MGIT MICs of linezolid in pure isolates of M. tuberculosis from 457 TB-PRACTECAL study participants disaggregated by country of enrolment is summarised in Figure 4.8. The median and mode MIC was 0.5mg/L with a range of 0.063 to 1mg/L. None of the isolates were above the linezolid critical concentration of 1mg/L (90).



	<0.063	0.125	0.25	0.5	1
Uzbekistan	0	0	24	186	17
South Africa	1	10	71	67	16
Belarus	0	0	7	49	9

Figure 4.8: Distribution of M.tb pre-treatment isolates across linezolid MICs in the TB-PRACTECAL trial

4.3.8. Probability of target achievement simulations

Simulating 2,000 virtual patients, the attainment of the combined bactericidal EC_{80} and resistance prevention $AUC_{0\text{-}24}$ /MIC target of 119, at the 600mg daily dose, only those with an MIC of 0.25mg/L or below had a probability above 90%. Attainment at the 300mg daily dose was one dilution lower at 0.125mg/L MIC as shown in figure 4.9. At 0.5 mg/L the PTA was 52.4% for 600mg dose and at 1mg/L and above the attainment was negligible (see table 4.9 below).

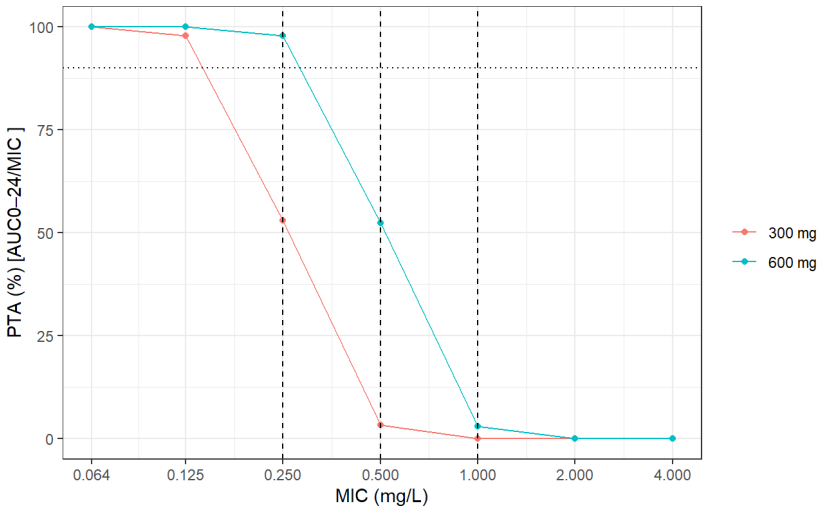


Figure 4.9: Plot of the probability of fAUC/MIC target attainment of 119.

Only at the bacteriostasis *f*AUC$_{0\text{-}24}$/MIC target of 16.24 was a PTA of above 90% achieved for all susceptible isolates for both doses as shown in table 4.9 and plots in appendix 5.

Table 4.5: Summary table of PTAs for the 600mg and 300mg daily doses for linezolid susceptible isolates

MIC	***f*AUC/MIC target attainment - 300mg, %**				***f*AUC/MIC target attainment - 600mg, %**			
	119	***73.6***	***43.47***	***16.24***	***119***	***73.6***	***43.47***	***16.24***
0.064 mg/l	100	100	100	100	100	100	100	100
0.125 mg/l	97.85	99.9	100	100	100	100	100	100
0.25 mg/l	53.05	92.35	99.85	100	97.9	100	100	100
0.5 mg/l	3.35	31.2	82.4	100	52.4	92.15	99.85	100
1 mg/l	0	0.7	17.5	96.50	3.1	30.15	82.85	100

Time above MIC was considered relevant later in treatment especially when resistant strains have developed in the hollow fibre study (78), in our study as shown in appendix 5, neither dose could have achieved the %*f*T>MIC target of 100% at a linezolid MIC of 0.06mg/L.

4.4. Discussion

A one-compartment disposition model with first order absorption and elimination best described the linezolid pharmacokinetics in rifampicin resistant tuberculosis patients from South Africa and Belarus being treated with BPaL-based short regimens. Fat-free mass allometric scaling and a Caucasian race covariate optimised the linezolid pharmacokinetics model. The primary parameters were a clearance of 5.88 L/hr, a volume of distribution of 58.5 L and a fixed absorption rate constant of 1.23 (fixed) hr-1. At a daily dose of 600mg, the median AUC$_{0\text{-}24}$ was 90.40 mg*h/L and at 300 mg daily the median AUC$_{0\text{-}24}$ was 45.67 mg*h/L. The linezolid median MIC in MGIT in the study population was 0.5 mg/L. The 600mg dose probability of *f*AUC$_{0\text{-}24}$ / MIC target of 119 was reached for MIC of 0.25 mg/L.

Most reported population pharmacokinetic models are one compartment but with varying approaches to the absorption including transit compartments (75, 83) or lag

functions. There was only one 2-compartment model (77) and some included an inhibition compartment to take into account autoinhibition in the elimination phase (83).

Linezolid clearance in TB patients from across the world has been reported to range between 3.81 (75) to 7.69 (84) L/hr with limited explanation in the variability. Our study's clearance falls within this range but is on the higher end at 5.88 L/hr. Recent studies have identified several covariates in their population pharmacokinetic models. The commonest being body size-related such as fat free mass, which we included in our model, and weight (77, 80, 83). Variation in kidney functioning such as creatinine clearance and BUN (80) have either been identified in model development (77) or have been included in allometric adjustment (86). Although creatinine clearance was identified in the first cycle at $p<0.05$, it was dropped in further steps in our model development (appendix able S3.1) which could have been influenced by the strict inclusion criteria for the trial, where patients with severely impaired renal function were excluded. Diabetes Mellitus type 2 has also been reported as a covariate (77), but this could have been explained by its effect on renal functioning. Similar to what has been reported elsewhere(85), HIV infection did not significantly influence our model as well. We identified race as influencing both volume and clearance but only retained Caucasian race on clearance in the final model, this may be explained by genetic variability (90) which was also reported in our study population; (91) Variant *3 in CYP3A5*3 was associated with lower linezolid trough values. Linezolid volume of distribution has been reported to range from the lowest at 31 litres (75) to the highest 50 litres (83, 87), we reported a volume close to the higher end.

We report the median AUC_{0-24} for the 300mg and 600mg daily doses as 46 mg*hr/L and 90 mg*hr/L respectively (table 4.3), these are up to 50% lower than exposures reported by some (38, 85) but higher than others (52).

The median baseline MIC of the study population was 0.5mg/L, with 93% of the isolates at this MIC or lower (table 4.8). This is similar to the distribution reported by Abdelwahab *et al*. (85) but at almost one dilution step higher than Zhang *et al*. (77). Half of the isolates from South Africa had an MIC equal to or below 0.25mg/L while for Belarus and Uzbekistan this was 11%. As this difference is just one dilution apart, and despite

stringent standards implemented in the trial these could be analytical differences as three different laboratories were used.

Although at 600mg daily dose only 25% of all PRACTECAL participants would have achieved the 119 AUC/MIC PKPD efficacy target, 50% of South African participants would have reached it. The achievement of the AUC/MIC PKPD efficacy target of 73.6, which was shown to sterilise media (78), was possible in 93% of the participants at a dose of 600mg daily. The 300mg dose is still useful as even at the linezolid critical concentration of 1mg/L, the bacteriostatic AUC/MIC PKPD target is achieved (table 4.9 and appendix figure S5.3).

The median C_{trough} of 0.71 mg/L (interquartile range: 0.41 - 1.22) for the 600mg dose and 0.39 mg/L for the 300mg dose (table 4.3) may explain the very low linezolid related adverse events reported in the TB-PRACTECAL trial (72) as a threshold of 2mg/L has been defined as associated with low toxicity (80, 85).

In the treatment of drug-resistant tuberculosis, linezolid 1200mg daily has been shown to be associated with significant toxicity (92), 600mg daily throughout treatment has been recommended by WHO (32) with concerns that reducing to 300mg dose is not useful(85). Little has been established on the role that linezolid has in a regimen such as BPaLM or BPaLC where the companion drugs may play different roles due to their variability in penetration of the different TB lesions (87).

The main limitation of the study is related to the fact that it was a sub-study, so the sample size was opportunistic and although the timing of samples was identified through an optimal design approach (79), the estimated total sample size was not achieved. Although the model included residual error parameters, it may not fully account for potential misreporting of drug intake as not all doses were observed by research staff.

4.5. Conclusion

Recognising that BPaLM, BPaLC and BPaL regimens are highly efficacious, our study provides explanatory evidence that even relatively low linezolid exposure may have contributed to these outcomes. These results reignite the need to explore combined PKPD efficacy and safety targets for linezolid and other new oxazolidinones when used as part of efficacious regimens.

4.6. Chapter 4 Supplementary appendices

4.6.1. Appendix 1: base model selection

Table S1: Type of algorithm, objective function values (OFV) and delta OFV of explored models.

	Model description	Algorithm	OFV	delta from run008	Comment
1	run001.focei | 1comp | linCmt | logn RUV	focei	13487.22	3810.51	
2	run002.focei | 1comp | ODE | logn RUV	focei	9729.79	53.08	
3	run003.focei | 1comp |linCmt | Add + Prop RUV	focei	22210.23	12533.52	
4	run004.focei | 1comp | ODE | Add + Prop RUV	focei	9980.43	303.72	
5	run005.focei | 1comp | ODE | Prop RUV	focei	9981.71	305.00	
6	run006.focei | 1comp | linCmt | Prop RUV	focei	7828105908	7828096231.25	very high OFV, failed convergence
7	run007.focei | 2comp | ODE | Prop RUV	focei	9963.25	286.54	
8	**run008.focei | 2comp | ODE | logn RUV**	focei	9676.71	-	lowest OFV
9	run009.focei | 2comp | ODE | Add + Prop RUV	focei	9954.9	278.19	
10	run0015.focei | 2comp | fixed ka | ODE | logn RUV	focei	9687.69	10.98	
11	run0016.focei | 1comp | fixed ka | ODE | logn RUV	focei	9718.85	42.14	

	Model description	Algorithm	OFV	delta from run008	Comment
1	run001.saem | 1comp | linCmt | logn RUV	saem	13999.12	4312.4	
2	run002.saem | 1comp | ODE | logn RUV	saem	9732.11	45.39	
3	run003.saem | 1comp | linCmt | Add + Prop RUV	saem	11654.07	1967.35	
4	run004.saem | 1comp | ODE | Add + Prop RUV	saem	10018.54	331.82	
5	run005.saem | 1comp | ODE | Prop RUV	saem	10019.48	332.76	
6	run006.saem | 1comp | linCmt | Prop RUV	saem	7828105283	7828095596	very high OFV, failed convergence
7	run007.saem | 2comp | ODE | Prop RUV	saem	10079.37	392.65	
8	**run008.saem | 2comp | ODE | logn RUV**	saem	9686.72	-	lowest OFV
9	run009.saem | 2comp | ODE | Add + Prop RUV	saem	10086.45	399.73	
10	run0015.saem | 2comp | fixed ka | ODE | logn RUV	saem	9688.58	1.86	
11	run0016.saem | 1comp | fixed ka | ODE | logn RUV	saem	9717.02	30.30	

Comp = compartments, add = additive, Prop = proportional, logn = logarithm, OFV= objective function value.

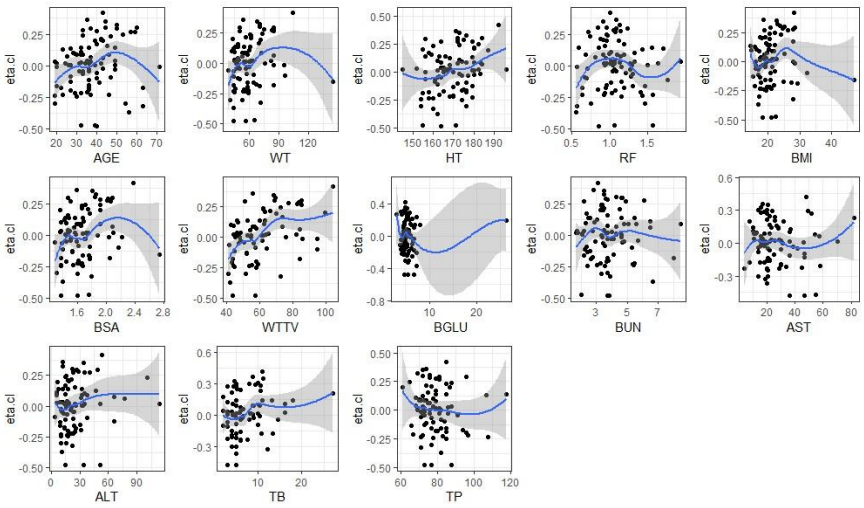
Run008.focei had the lowest OFV (9676.71), followed by run0015.focei (ΔOFV +10.98), run0016.focei (ΔOFV +42.14) and run002.focei (ΔOFV +53.08).

Table S2: Base model parameter estimates

Parameter		Est.	SE	%RSE	Back-transformed(95%C	BSV(CV%)	shrink(SD)%
tka	1/hr	0.207	FIXED	FIXED	1.23		
tcl	L/hr	1.89	0.0524	2.77	6.62 (5.98, 7.34)	23.4	13.2%<
tv	L	4.07	0.0616	1.51	58.8 (52.1, 66.3)	12.7	34.6%>
logn.sd		0.89			0.89		

4.6.2. Appendix 2: Graphical analysis - visual covariate exploration

Parameter exploration matrix – continuous variables against eta.cl.



WT = weight, HT = height, RF = Creatinine clearance, BMI = body mass index, BSA = body surface area, WTTV = time varying weight, BGLU = baseline glucose, BUN = blood urea nitrogen, AST = aspartate transferase, ALT = alanine transferase, TB = total bilirubin and TP = total protein

Figure S2.1: Continuous covariates of study population plotted against the eta clearance

Parameter exploration matrix – continuous variables against eta.v

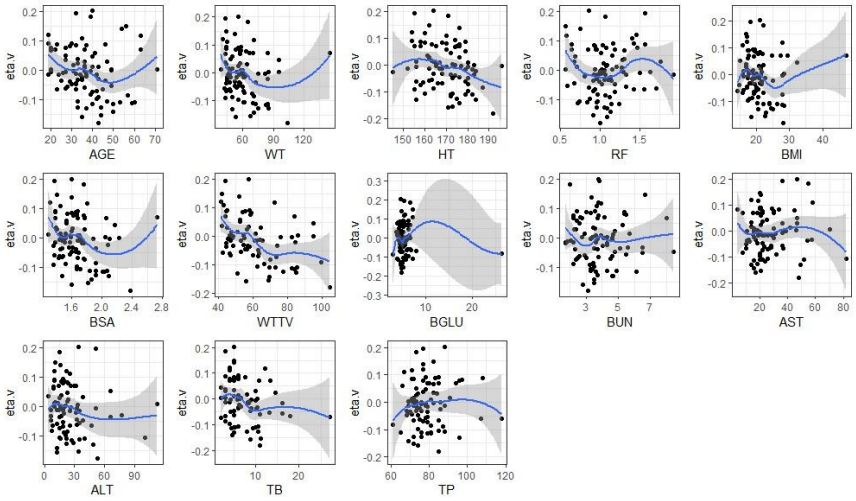


Figure S2.2: Continuous covariates of study population plotted against the eta volume of distribution

Parameter exploration: categorical covariates against ETAs

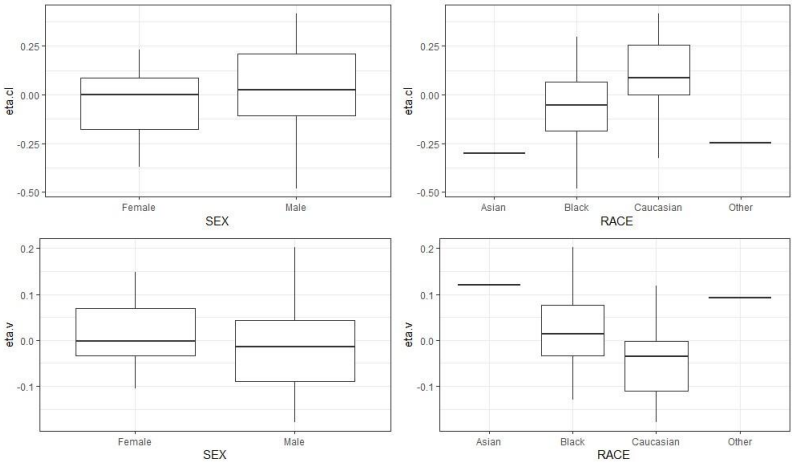
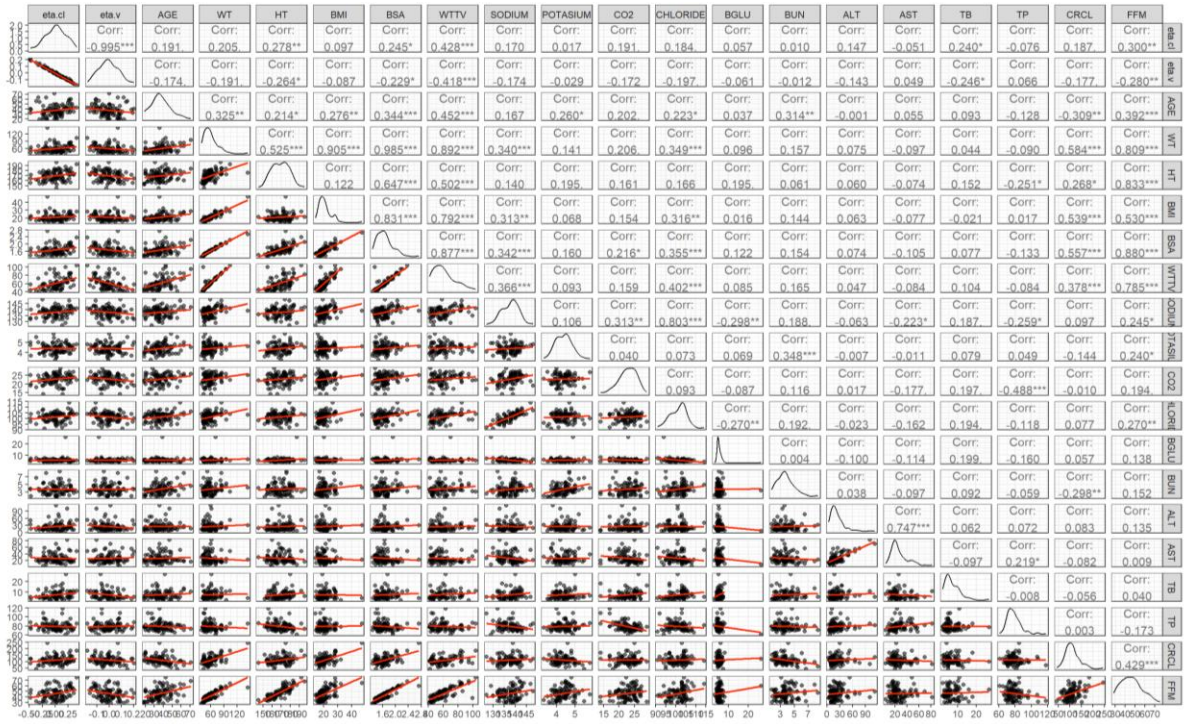


Figure S2.3: Categorical covariates of study population plotted against the etas of clearance and volume of distribution

Covariate exploration matrix: covariates collinearity and ETAs correlation coefficient.



eta.cl = eta on clearance, eta.v = eta on volume of distribution, CRCL = creatinine clearance, FFM = fat-free mass

Figure S2.4: covariate matrix of scatter plots of selected covariates against each other to explore collinearity and with a regression line in red and the upper triangle showing the calculated correlation coefficients.

4.6.3. Appendix 3: covariate model development

Cycle 1:

Individual models for each selected covariate as above was ran in nlmixr2. The weight, FFM and BMI covariates were allometrically adjusted. The FFM code was as below:

```
# fit.ffm <- "basemodel" %>%
#model({cl <- exp(tcl + eta.cl+ logFFM*covffmPow1)}) %>%
#model({v <- exp(tv + eta.v+ logFFM*covffmPow2)}) %>%
#ini(covffmPow1= fix(0.75)) %>%
#ini(covffmPow2= fix(1)) %>%
#nlmixr(est="focei",control = foceiControl(seed = 1234,print = 5),
#table=list(censMethod = "cdf",cwres=TRUE, npde=TRUE))
```

The objective function values of the cycle 1 models output were tabulated as below. Using the base model OFV as reference, delta OFV was calculated as:

ΔOFVc1 = Cov1_base OFV - covariate model OFV

Table S3.1: Results of Cycle 1 covariate model exploration

	Model	OFV	Delta OFV_{c1}
1	Cov1_base	9716.564	0.00000000
2	Cov2_age_cl	9711.972	-4.59193059
3	Cov3_weight_cl	9716.543	-0.02068284
4	Cov4_bmi_cl	9718.528	1.96452425
5	Cov5_FFM_cl	9712.408	-4.15620965
6	Cov6_BUN_cl	9716.504	-0.06021092
7	Cov7_ALT_cl	9714.741	-1.82242893
8	Cov8_AST_cl	9716.366	-0.19826418
9	Cov9_ClCr_cl	9712.639	-3.92506233
10	Cov10_Total Protein_cl	9716.018	-0.54568003
11	Cov11_sex_cl	9713.296	-3.26776531
12	Cov12_race_caucasian_cl	9698.968	-17.59582533
13	Cov13_race_black_cl	9704.341	-12.22256742
14	Cov14_HIV_cl	9715.492	-1.07216884
15	Cov15_BPaLM_cl	9715.774	-0.78981965
16	Cov16_BPaLC_cl	9716.260	-0.30417675
17	Cov17_BPaL_cl	9716.502	-0.06200468
18	Cov18_age_v	9715.166	-1.39796456
19	Cov19_Total Protein_v	9716.455	-0.10920054
20	Cov20_sex_v	9716.152	-0.41205291
21	Cov21_race_caucasian_v	9702.174	-14.39032888
22	Cov22_race_black_v	9705.954	-10.60963216
23	Cov23_HIV_v	9716.202	-0.36151629
24	Cov24_BPaLM_v	9715.917	-0.64737355
25	Cov25_BPaLC_v	9716.496	-0.06786253
26	Cov26_BPaL_v	9716.876	0.31176857

Adding FFM allometry, age, creatinine clearance (CRCL) Caucasian and black race on clearance and Caucasian and black race on volume improved the model fit significantly at p <0.05 as seen with ΔOFVc1s in table S3.1 of above 3.84.

Cycle 2

The covariates selected in cycle 1 and renal and hepatic function-related covariates were further individually added to an FFM allometry base model and ran in nlmixr2. The model integrating FFM allometry and Caucasian race was as follows:

```
# fit. ffm.caucasian <- "FFMallometry basemodel" %>%
#  model({cl <- exp(tcl + eta.cl+ logFFM*covffmPow1+ RACECAUCASIAN*beta.caucasian)}) %>%
#  ini(beta.caucasian= 1) %>%
#  nlmixr(est="focei",control = foceiControl(seed = 1234,print = 5),
#      table=list(censMethod = "cdf",cwres=TRUE, npde=TRUE))
```

The objective function values of the Cycle 2 models output were tabulated as below. Using the FFM allometry model OFV as reference, delta OFV was calculated as:

ΔOFVc2 = Cov27_FFM_cl OFV - covariate model OFV

Table S3.2: Results of Cycle 2 covariate model exploration

	Model	OFV	Delta_OFV$_{c2}$
1	Cov1_base	9716.564	4.15620965
2	Cov27_FFM_cl	9712.408	0.00000000
3	Cov28_FFM.age_cl	9709.071	-3.33648625
4	Cov29_FFM.BUN_cl	9712.390	-0.01781191
5	Cov30_FFM.ALT_cl	9710.979	-1.42859561
6	Cov31_FFM.AST_cl	9712.101	-0.30671357
7	Cov32_FFM.ClCr_cl	9709.514	-2.89328047
8	Cov33_FFM.Total Protein_cl	9712.002	-0.40530067
9	Cov34_FFM.sex_cl	9710.928	-1.47972710
10	Cov35_FFM.race_caucasian_cl	9696.057	-16.35043412
11	Cov36_FFM.race_black_cl	9700.882	-11.52611596
12	Cov37_FFM.HIV_cl	9711.283	-1.12419170

13	Cov38_FFM.BPaLM_cl	9711.463	-0.94471564
14	Cov39_FFM.BPaLC_cl	9711.957	-0.45071830
15	Cov40_FFM.BPaL_cl	9712.289	-0.11913310
16	Cov41_FFM.age_v	9709.806	-2.60159182
17	Cov42_FFM.Total Protein_v	9712.292	-0.11522914
18	Cov43_FFM.sex_v	9711.066	-1.34149523
19	Cov44_FFM.race_caucasian_v	9696.133	-16.27420700
20	Cov45_FFM.race_black_v	9700.460	-11.94722291
21	Cov46_FFM.HIV_v	9711.952	-0.45611505
22	Cov47_FFM.BPaLM_v	9711.685	-0.72264021
23	Cov48_FFM.BPaLC_v	9712.142	-0.26552197
24	Cov49_FFM.BPaL_v	9712.497	0.08927648

Caucasian and Black race on clearance, Caucasian, and black race on volume with a reduction in the objective function value (ΔOFVc2) of greater than 3.84 (at a p value of <0.05) were considered to significantly improve the model fit as seen in table S3.2 above.

Cycle 3

The covariates selected in cycle 2 were individually added to the best performing model - FFM allometry.race_caucasian as a base model and ran in nlmixr2.

Table S3.3: Results of Cycle 3 covariate model exploration

	Model	OFV	Delta OFV
1	Cov1_base	9716.564	20.506644
2	Cov27_base.cycle1_FFM_cl	9712.408	16.350434
3	Cov35_base.cycle2_FFM.race_caucasian_cl	9696.057	0.000000
4	Cov50_FFM.race_caucasian.black_cl	9694.123	-1.934120
5	Cov51_FFM.race_caucasian.black_v	9698.548	2.490464

No model improved significantly with the addition of a third covariate as seen in table S3.3.

No specific backward elimination cycle was performed as Cov35__FFM.race_caucasian_cl had already demonstrated a drop in OFV of 16.35 from Cov27_FFM_cl. Concluding that adding the covariate - Caucasian race to the base model, improves the model fit significantly, at a p value of <0.01 and p < 0.001.

4.6.4. Supplementary appendix 4: Graphical analysis of final model

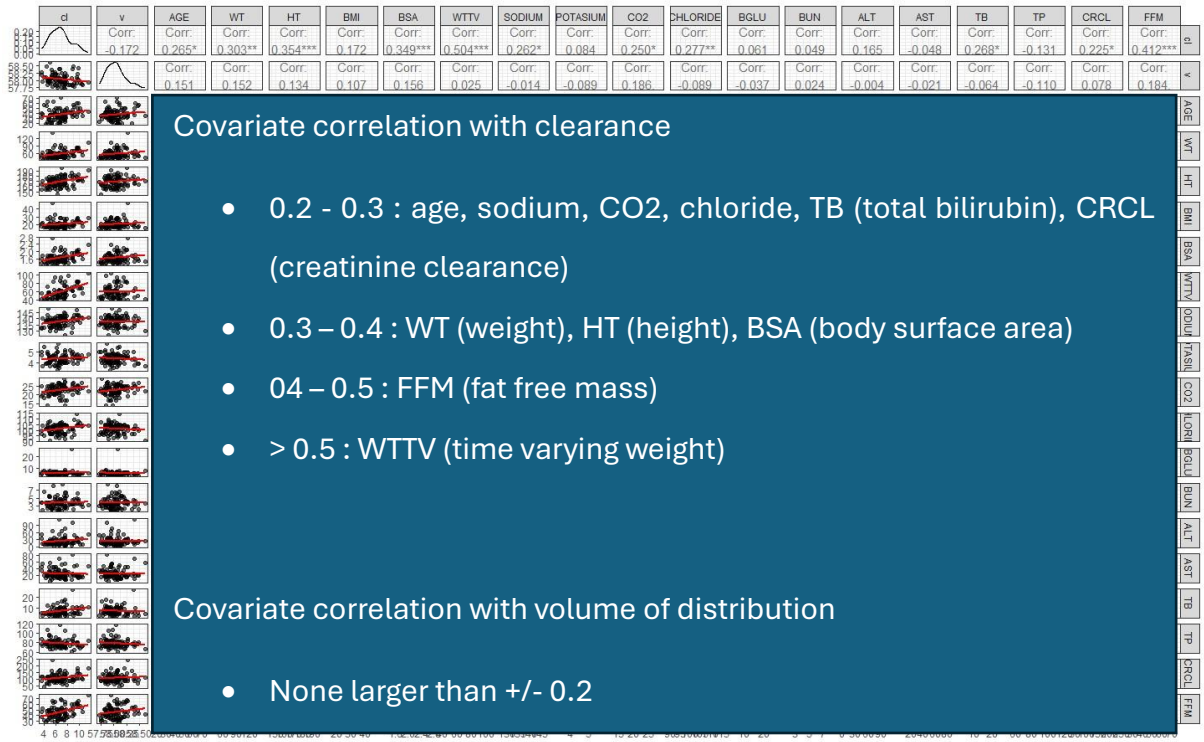
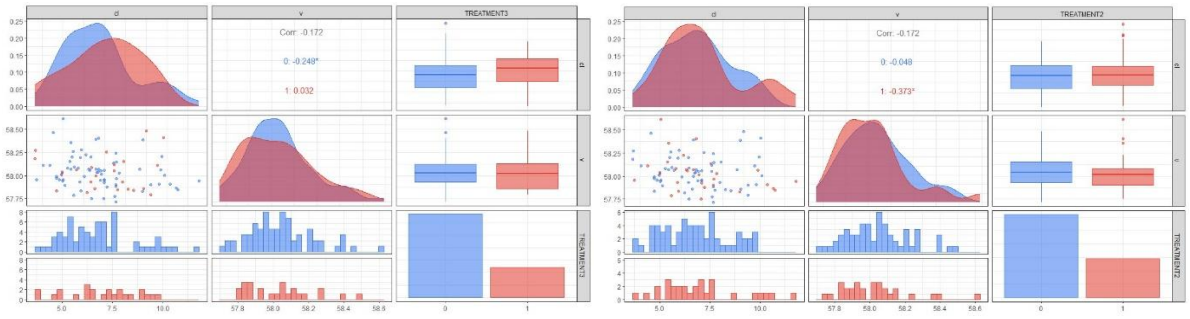


Figure S4.1: covariate matrix in final model

Treatment regimen, sex and HIV status categorical covariates did not show any observed differences in both apparent clearance and volume of distribution (figure 4.16).



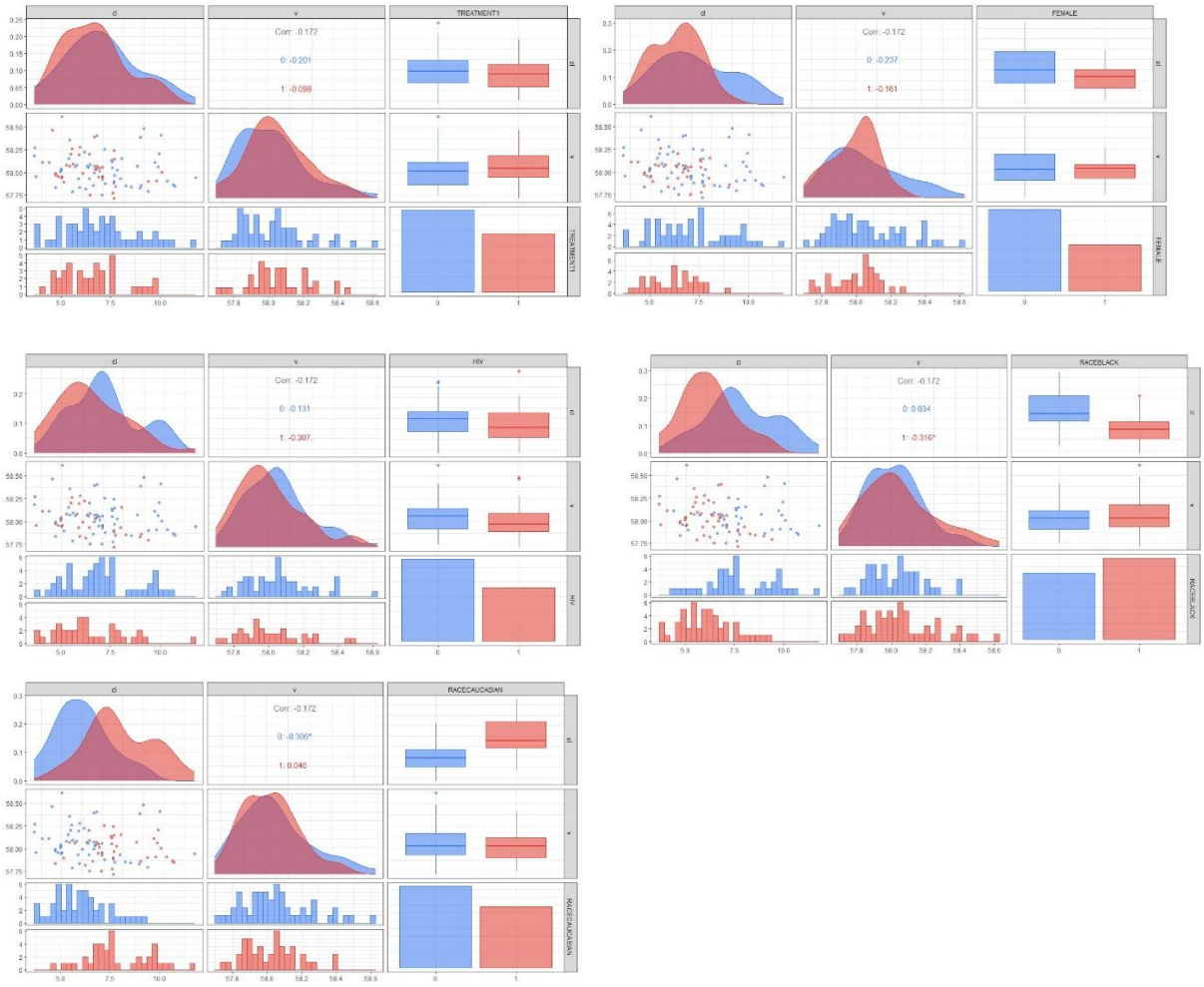


Figure S4.2: categorical covariate plots of the final model

4.6.5. Appendix 5 Probability of target attainment

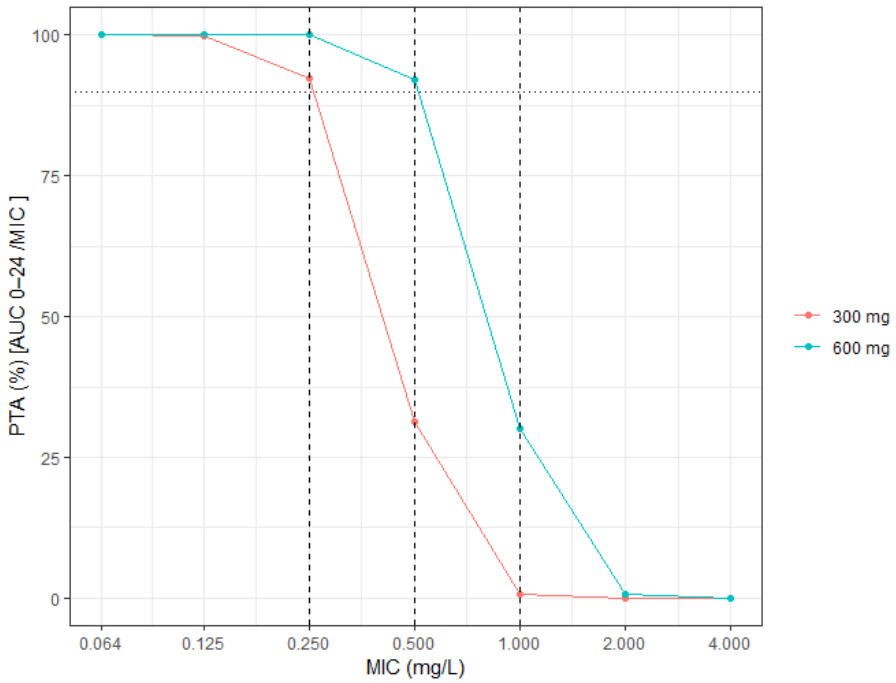


Figure S5.1: Plots of the probability of *f*AUC/MIC target attainment of 73.6.

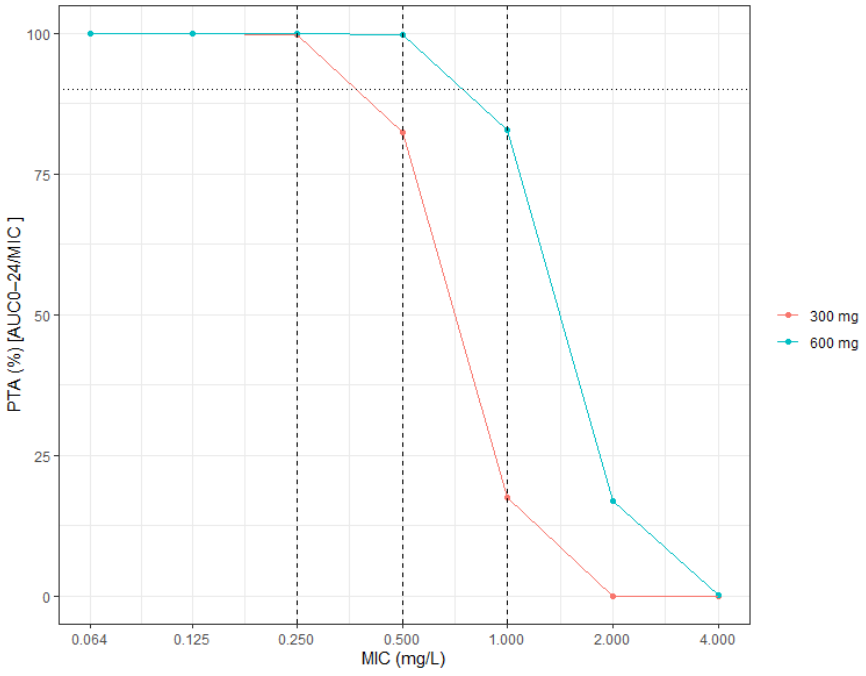


Figure S5.2: Plots of the probability of *f*AUC/MIC target attainment of 43.47

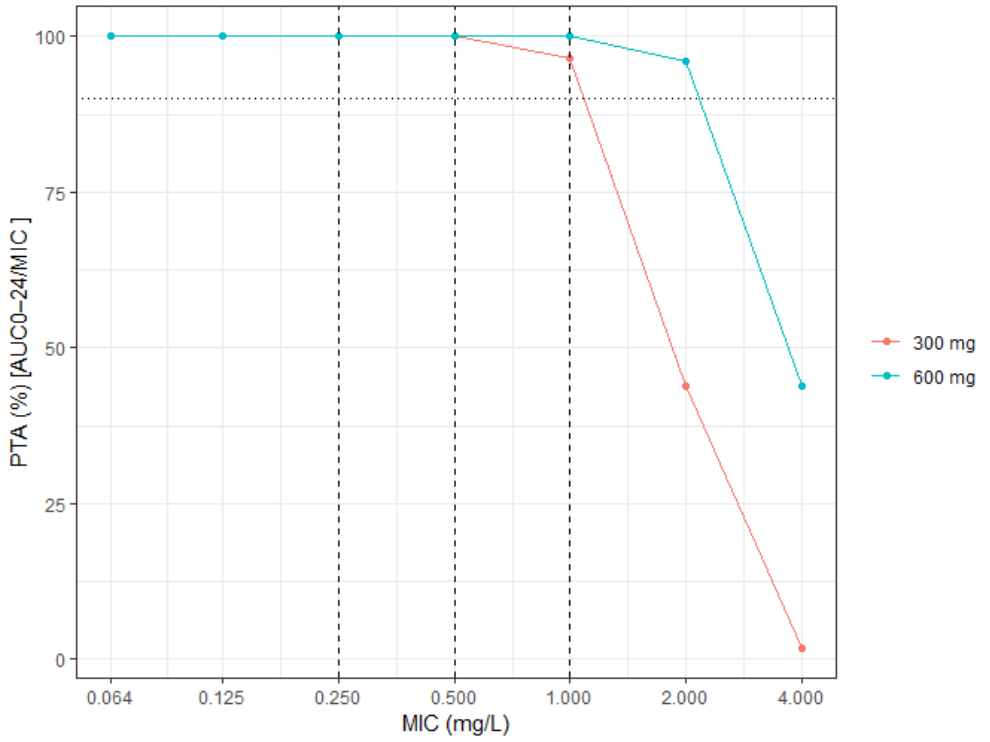


Figure S5.3: Plots of the probability of *f*AUC/MIC target attainment of 16.24

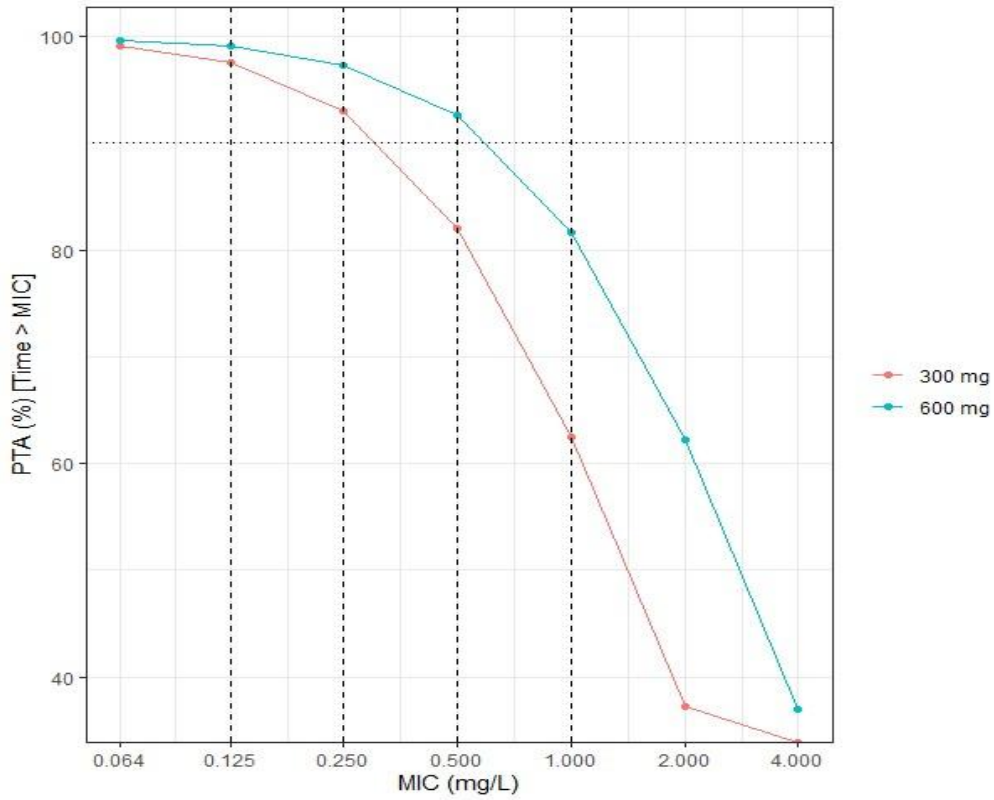
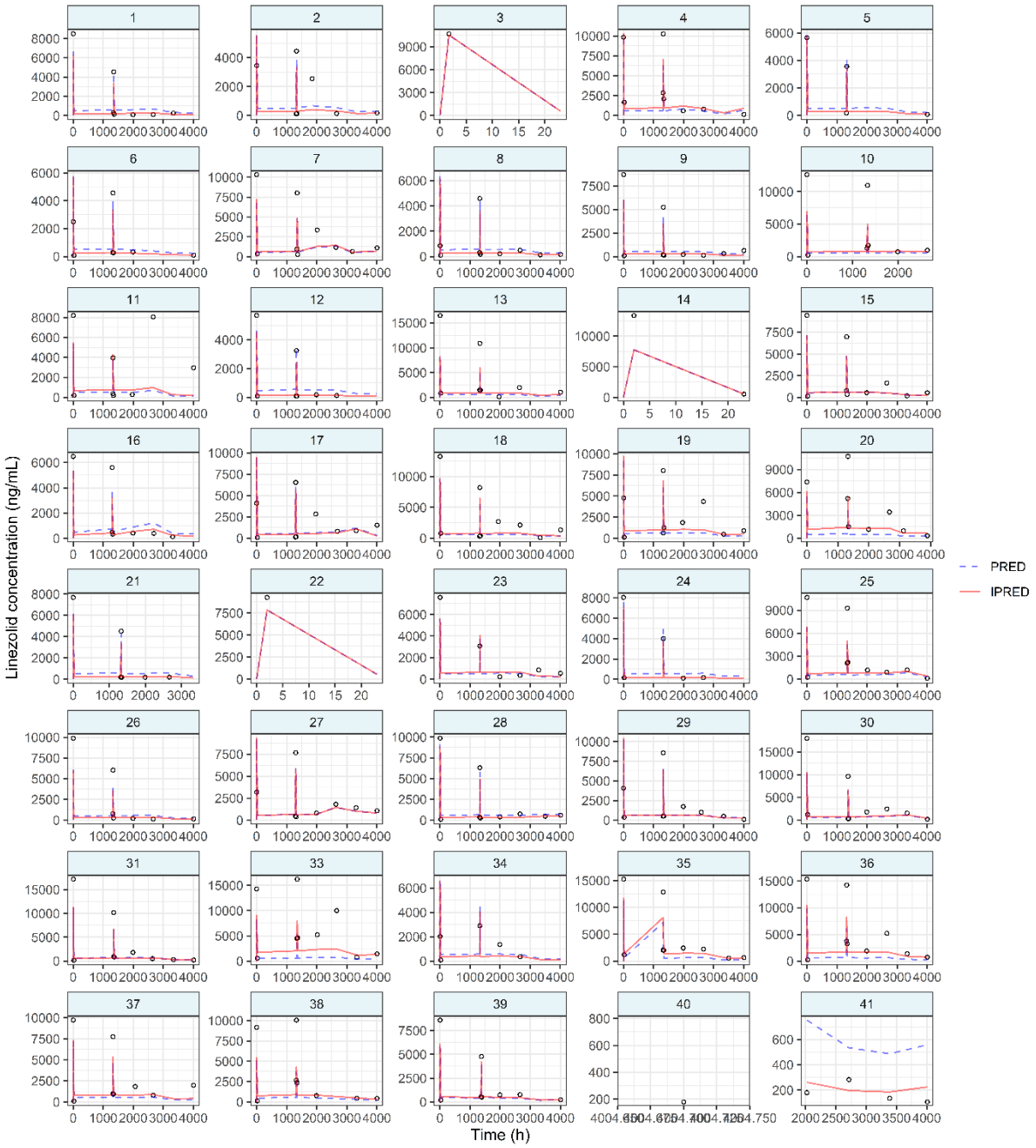
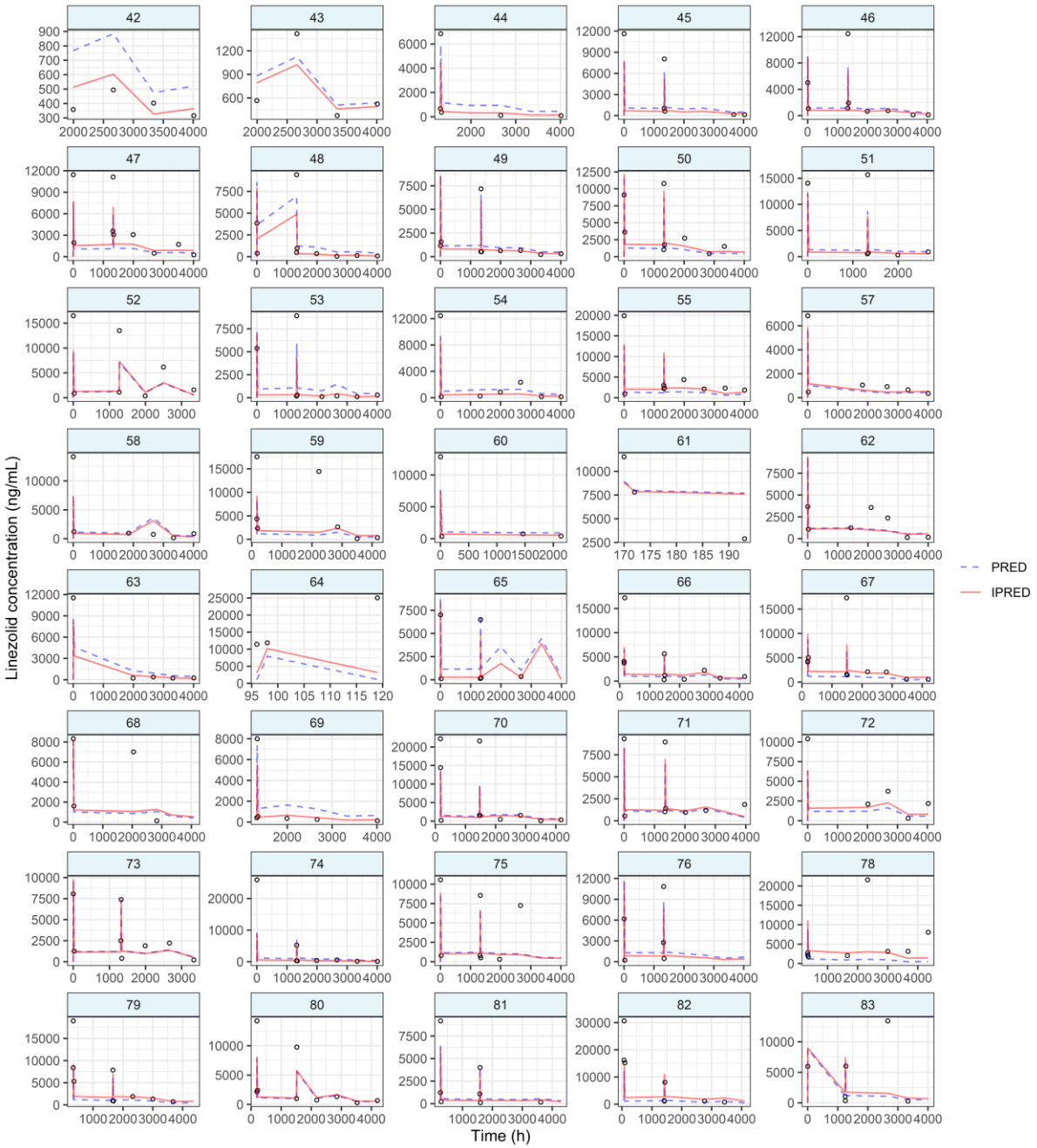
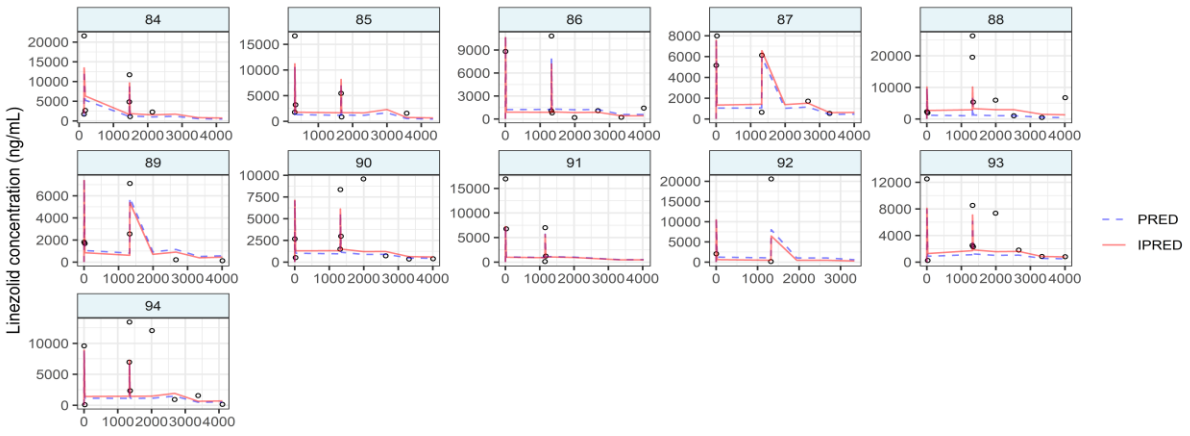


Figure S5.4: Probability of %*f*T>MIC target attainment

4.6.6. Appendix 6: Individual drug profiles




Linezolid concentration (ng/mL)
Time (h)
PRED
IPRED


Linezolid concentration (ng/mL)
PRED
IPRED

Time (h)

4.6.7. Appendix 7: Used r packages

```
library(rxode2)

library(nlmixr2)

library(reshape2)

library(ggplot2)

library(tidyverse)

library(PerformanceAnalytics)

library(psych)

library(dplyr)

library(GGally)
```

4.6.8. Appendix 8: R code for final model

```
lzd.mod <- function() {
          ini({
           tka <- fix(0.207014169384326)
           label("1/hr")
           tcl <- 1.7709838100771
           label("L/hr")
           tv <- 4.06903212913131
           label("L")
           logn.sd <- c(0, 0.888277644306636)
           covffmPow1 <- fix(0.75)
           covffmPow2 <- fix(1)
           beta.caucasian <- 0.275778069209016
           eta.cl + eta.v ~ c(0.0654073686493719, 0.00177971008533524,
                              0.00319721177180204)
            })

 model({
                    ka <- exp(tka)
          cl <- exp(tcl + eta.cl + logFFM * covffmPow1 + RACECAUCASIAN *
beta.caucasian)
           v <- exp(tv + eta.v + logFFM * covffmPow2)
           k <- cl/v
          d/dt(depot) = -(ka * depot)
           d/dt(centr) = (ka * depot) - (k * centr)
           cp = centr/v
           cp ~ logn(logn.sd)
           })
        }
```

Chapter 5: Pretomanid population pharmacokinetics and probability target attainment in participants treated with BPaL based regimens for rifampicin-resistant tuberculosis.

5.1. Introduction

This chapter reports on the study methods and results of the population pharmacokinetics of pretomanid and its probability target attainment. The results section describes the participants of the study, the pharmacokinetic data that was used and the population pharmacokinetic model building. The intermediate model building steps detail out the structural, statistical and covariate model building and evaluation of the final linezolid population pharmacokinetic model. The parameter estimates derived from the final model are presented, including a discussion on how they compare with those from previously published papers. Pretomanid MICs in the TB-PRACTECAL clinical trial (72) are presented and used in interpreting the probability target attainment analyses. Finally, a discussion of the role of these results in the cumulating evidence around pretomanid use in the treatment of tuberculosis is presented.

5.2. Methods

5.2.1. Study design

This was a sub study nested in the TB-PRACTECAL randomised controlled trial in patients with rifampicin resistant tuberculosis. Participants received one of three investigational regimens. BPaL arm consisted of bedaquiline 400mg daily for 2 weeks then 200mg three times a week for 22 weeks, pretomanid 200mg daily for 24 weeks and tapered dose linezolid 600mg daily for 16 weeks then 300mg for 8 weeks. Clofazimine 100mg daily for 24 weeks was added in BPaLC arm or Moxifloxacin 400mg daily for 24 weeks in BPaLM arm. Blood samples were collected on Day 1 (0, 2 and 23 hours), Weeks 8 (predose, 6.5 and 23 hours), 12, 16, 20, 24, 32 and 72 post randomisation visits. Drug concentrations were quantified in a GCP laboratory using a high-performance liquid chromatography-tandem mass spectrometry. The lower limit of quantification for pretomanid was 7ng/mL.

5.2.2. Pharmacometric analysis

nlmixr2, an open-source R package was used for population PK modelling and simulation estimation. R v4.1.2 was used for dataset creation, data exploration and generation of tables and plots. A list of packages used are in appendix 1. The PopPK for pretomanid was analysed using a non-linear mixed effect modelling approach. The first-order conditional estimation with interaction (FOCE-I) algorithm in nlmixr2 was used. Inter-individual variability (IIV) at the parameter level and residual variability (RV) at the observation level made up the mixed effects analysis.

5.2.3. Structural model

The PopPK study first explored basic model structure based on the observed plasma concentration data. One-, two- compartment linear models were evaluated respectively with combined, proportional and additive residual error models. Finally, random effects on clearance (CL) and volume of distribution (V) without correlation were included in the model. A log-transformed residual error model was also tested. Various absorption models were explored including transit compartment models and fixed absorption constant (ka).

5.2.4. Covariate model

A covariate matrix of age, sex, weight, BMI, FFM, race, BUN, ALT, AST, TP, CLCR, treatment regimen and eta estimates on clearance and volume of distribution from the base model explored correlation as well as covariate collinearity. Allometric scaling was applied to both volume of distribution and clearance. The coefficients of the power model were fixed to 1 for V and 0.75 for CL. The selected covariates underwent stepwise forward inclusion ($P<0.05$, $\Delta OFV > 3.84$) and backward elimination ($p<0.001$, $\Delta OFV > 10.83$) to select those that would improve the model fit significantly.

5.2.5. Model evaluation

Goodness-of-fit plots were used to assess how well the model predicted individual and population values closely matched the observed PK data. Model validation was also

performed using visual predictive check (VPC) plots. The shrinkage, relative standard error, and variability value including omega and sigma value were also used to assess the precision and robustness of the model.

5.2.6. MIC

Minimum inhibitory concentrations were determined from a routine testing concentration set (1, 0.5, 0.25, 0.125, 0.063, 0.032 mg/L) in MGIT; testing was performed using a higher (8, 4, 2 mg/L) or lower (0.016 mg/L) testing concentration set if required. The results from all participants from the TB-PRACTECAL trial were summarised by country of enrolment and the median and interquartile range reported. Previously published pretomanid MIC data was used where PTA targets used a different methodology to MGIT.

5.2.7. Probability of Target Attainment

The probability of pretomanid pharmacokinetic-pharmacodynamic (PK/PD) target attainment at the early bactericidal activity (EBA) studied doses of 50mg, 100mg, 150mg and PRACTECAL dose of 200mg daily were simulated using the Monte Carlo methodology. Pretomanid maximum efficacy is achieved with a daily dose of 200mg, increased toxicity but not increased efficacy is experienced with higher doses in early bactericidal studies (93); doses of 50mg, 100mg and 150mg have captured the incremental efficacy and toxicity better (94). At a dose interval of less than 48 hours, pretomanid efficacy PK/PD can be defined by area under the free drug concentration curve (*f*AUC/MIC) or cumulative percentage of the dosing interval that the free drug concentration exceeds the MIC (*f*T>MIC) parameters (95). However, pretomanid in vivo protein binding has not yet been determined, approaches have included using total drug concentration (96), 85% binding (49) or a range of values between 85% and 95% binding (95).

The area under the free drug concentration curve ($fAUC_{0\text{-}24}$) of 2,000 virtual participants was used to calculate the probability of attaining *f*AUC/MIC target of 167, which is associated with >2 log10 reduction in CFU counts. The *f*T%>MIC targets required for bacteriostatic, 1-log10 kill and 1.59- log10 kill (80% maximum effect) which were 22%,

48% and 77%, respectively were also reported (49). Scenarios with assumed free drug proportions of 5%, 10% and 15% were analysed for each PKPD target.

5.3. Results

5.3.1. Study population

Table 5.1: baseline characteristics of study participants (n = 94)

Characteristic	Total
Female, n (%)	34 (36)
Age, median years (range)	36 (19-71)
Race, n (%)	
Asian	1 (1)
Black	52 (55.3)
Caucasian	40 (42.6)
Other	1 (1)
HIV status, n (%)	
positive	39 (41.5)
negative	54 (57.4)
not known	1 (1.1)
Regimen, n (%)	
BPaLM	28 (40.4)
BPaLC	30 (31.9)
BPaL	26 (27.7)
Weight, kg	56.8 (39.2 – 144.4)
Height, cm	170 (145 – 196)
BMI, Kg/m2	19.7 (13.3 – 47.2)
Fat Free Mass, kg	45.5 (28.6 – 75.5)
BUN (mmol/L)	3.6 (1.7 – 8.5)
ALT (IU/L)	19.5 (4 – 113)
AST (IU/L)	22 (4 – 82)
ALP (IU/L)	67 (37 – 132)
Albumin* (g/L)	44 (36 – 49)
Total protein* (g/L)	77 (61 – 118)
Creatinine (mcrmol/L)	66 (35 – 111)
Creatinine clearance (mL/min)	105.4 (43.4 – 243.8)

Median (min-max) if not stated otherwise. * n=39

BPaLM = bedaquiline+pretomanid+linezolid+moxifloxacin, C=clofazimine,

BMI= body mass index, ALT= alanine transaminase, AST= aspartate aminotransferase, ALP=alkaline phosphatase, BUN= blood urea nitrogen

94 study participants (36% female) with a median age of 36 years (range: 19 – 71 years), see table 5.1, contributed 952 timed plasma samples which upon bioanalysis were included in the pretomanid PopPK dataset. 86 samples were collected before the first dose and 866 samples after the first dose. 234 samples were deemed to be below the limitation of quantification, of which 151 samples were collected after treatment completion so only 9.5% were BLQ samples during treatment.

The observed concentrations of pretomanid ranged from 19.1 to 11,566.4 ng/ml. The median trough concentration was 1,788.94 ng/ml, with an interquartile range of 1,126.42 – 2,688.51 ng/ml (see Figure 5.1).

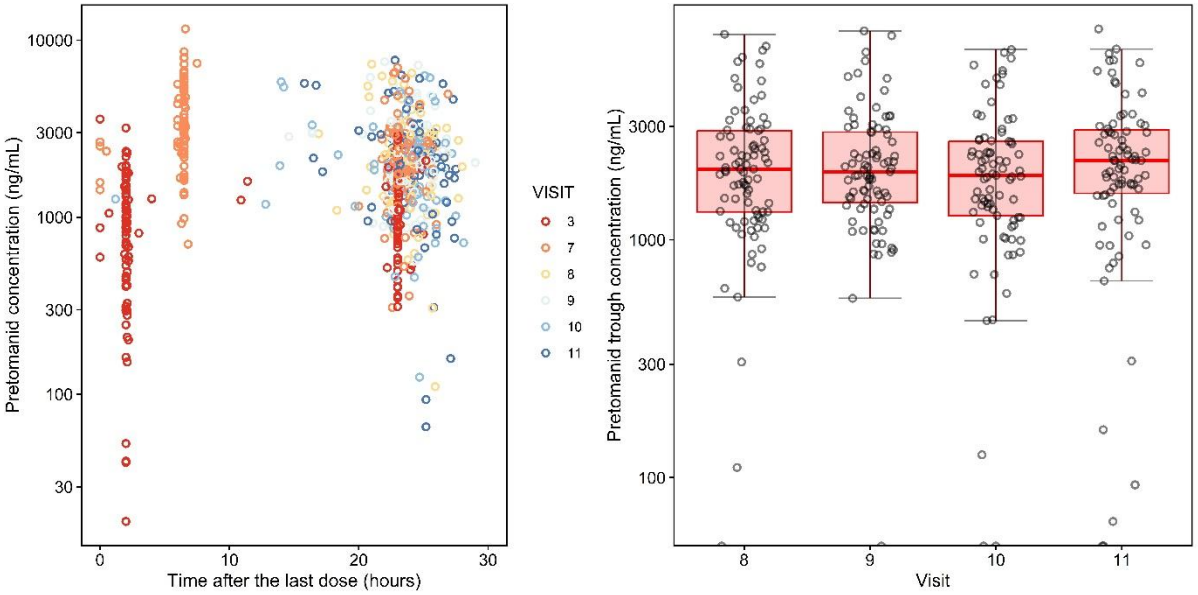


Figure 5.1: Plots of observed pretomanid concentrations by time after last dose (left – colour disaggregated by visit number)) and trough concentrations by time after first dose aggregated by study visit number (right – weeks 12, 16, 20, 24). The pink box represents the interquartile range with the horizontal red line representing the median while the whiskers represent the 5th and 95th percentiles.

The observed pk plots for each individual patient are shown in supplementary appendix 6.

5.3.2. Structural and variability model

A one-compartment first order absorption and elimination model with an OFV of 11695.91 was selected as the best to characterise the pretomanid observed PK data.

Although a two- compartment model had a lower objective function value than the one compartment model (ΔOFV = -4.53), it had worse estimation precision with RSE of clearance higher than 100%. Random effects on clearance and central volume of distribution were included in the model to explain the inter-individual variability. Combined residual error model was used for the unexplained variability as it performed better than additive (OFV = 12023.42), and proportional (failed to converge) models. The summary of model evaluation results with OFVs and delta ΔOFVs is in supplementary appendix 4.

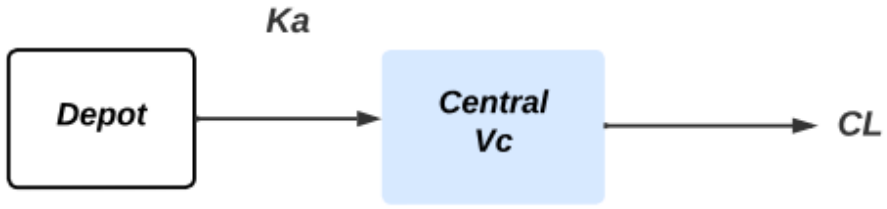


Figure 5.2: Schematic representation of the structural pretomanid model. Ka = absorption rate constant, Vc = central compartment, CL = clearance

The base model's r code is in appendix 2 and the ordinary differential equations describing the base models are below [5.1]:

$$\frac{dA_{depot}}{dt} = -K_a \times A_{depot}(t)$$

$$\frac{dA_{central}}{dt} = K_a \times A_{depot} - \frac{CL/F}{V_c/F} \times A_{central}(t)$$

where A_{depot} is the amount of pretomanid in the depot compartment. where $A_{central}$ is the amount of pretomanid in the central compartment. K_a is the absorption rate constant for the transfer of pretomanid from depot compartment to central compartment. CL/F is the apparent clearance of pretomanid. Vc/F is the apparent volume of distribution of pretomanid.

5.3.3. Covariate model

Allometric fat free mass on clearance and volume of distribution (ΔOFV -19.2 from selected base model) was the only covariate retained in backward elimination ($p < 0.001$). BUN, Creatinine clearance, total protein, AST, race, HIV infection, female sex and

treatment regimen were included in the forward first step analysis ($p < 0.05$), none of these were significant in the backward step. The summary of the covariate models' evaluation results with OFVs and delta ΔOFVs is in supplementary appendix 5.

5.3.4. Final model evaluation

Goodness-of-fit plots for the final PopPK model showed no significant bias from the unity line in both PRED VS DV and IPRED VS DV, indicating that the model predicted individual and population values closely matched the observed PK data (Figure 5.3).

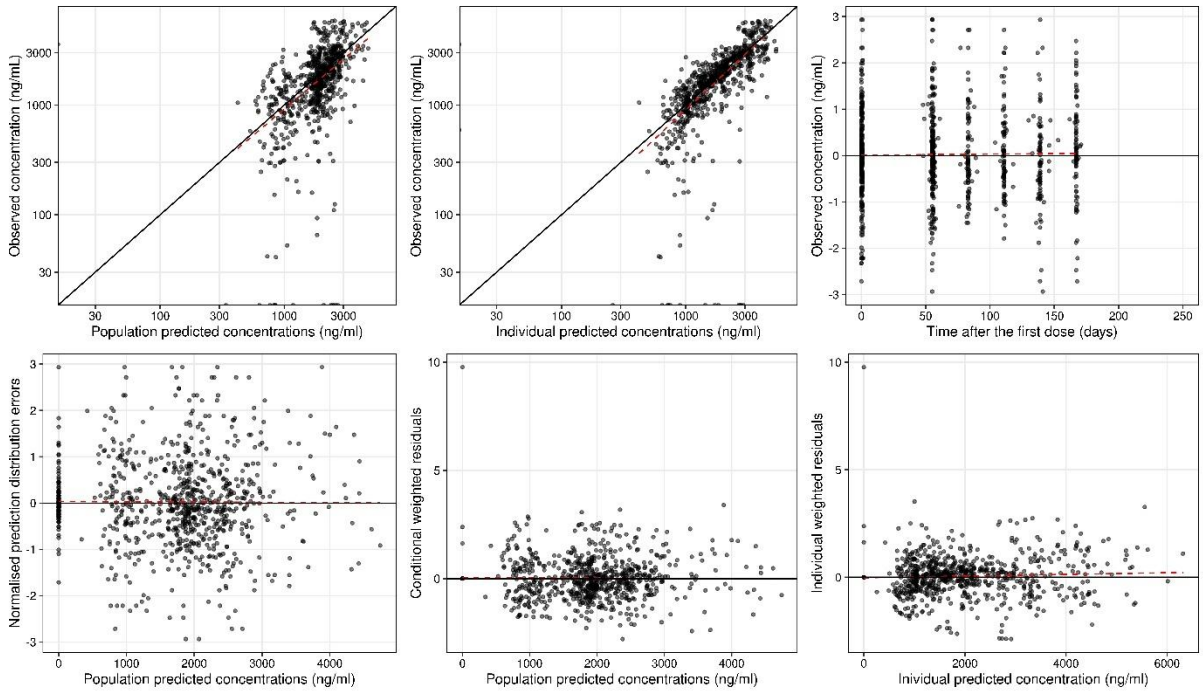


Figure 5.3: Final pretomanid model goodness of fit plots clockwise from top left: DV vs PRED, DV vs IPRED, DV vs TAFD, IWRES vs IPRED, CWRES vs PRED, NPDE vs PRED

Model validation using a visual predictive check (VPC) plot visually confirmed the predictive accuracy of the final model (figure 5.4).

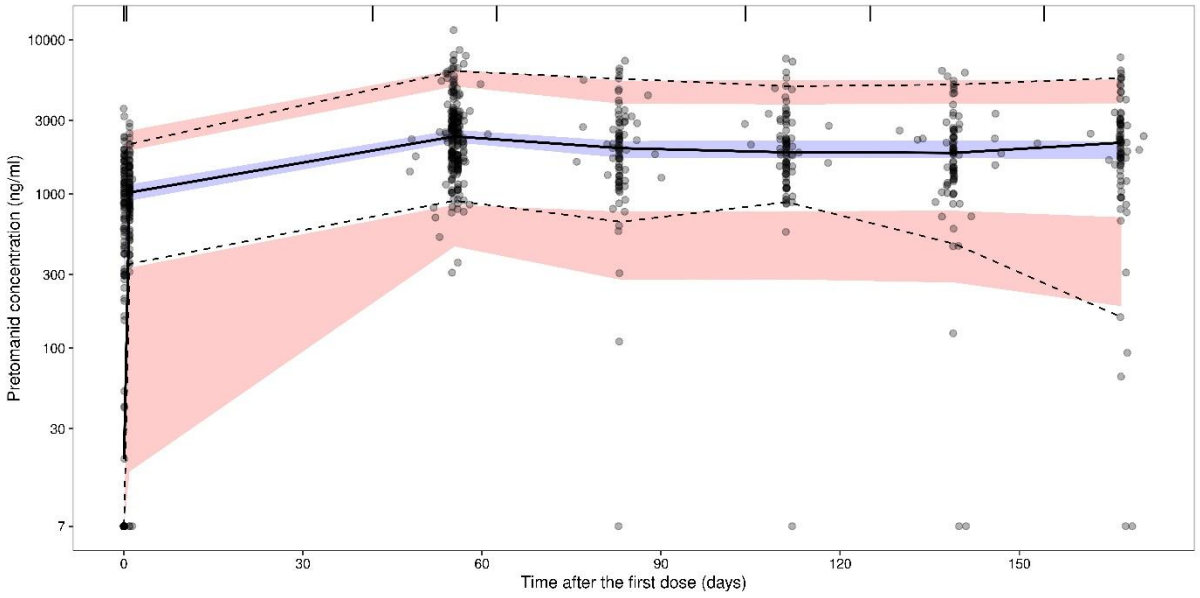


Figure 5.4a: Pretomanid final model visual predictive check. The black circles in the figure represents the observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. The purple area represents a simulation-based 95% confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink.

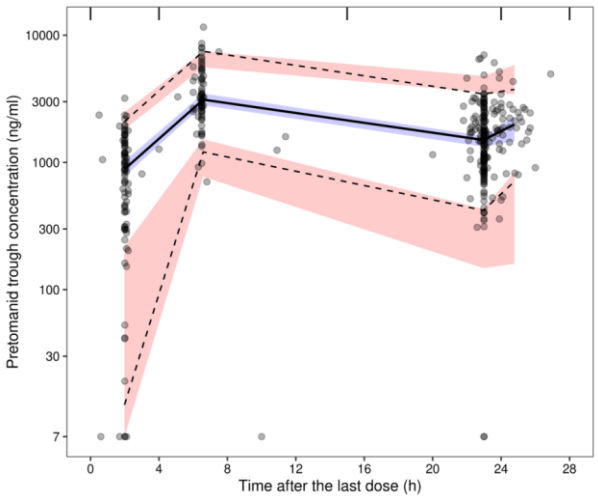


Figure 5.4b: Pretomanid final model visual predictive check. The black circles in the figure represents the observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. The purple area represents a simulation-based 95 % confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink.

The final model parameters are shown in table 5.2.

Table 5.2: Final pretomanid model primary and secondary parameter estimates

Parameter	Parameter estimate		
	Estimate (%RSE)	**95% CI**	**Shrinkage %**
Fixed effects parameters			
Ka, hr-1	0.316 (13.6)	0.233 – 0.429	
Cl, L/hr	3.08 (3.36)	2.86 – 3.32	
θ_{FFM}, Cl	0.10		
V, L	103 (2.02)	85.6 - 124	
θ_{FFM}, V	0.12		
Random effect parameters			
η(cl), %	32.9	-	9.65
η(v), %	33.6	-	36.7
Residual error parameters			
Proportional error	0.322	-	-
Additive error, mg/L	0.368	-	-
Secondary parameter estimates			
	Median	Interquartile range	Range
AUC_{0-24} (mg hr/L)	63733	49253– 87318	30853– 138179
C_{trough} (mg/L)	1965	1420 – 2767	65– 7702
C_{max} (mg/L)	3185	2551– 4217	1382– 6351

θ_{FFM}, CL = FFM theta on clearance, η(cl) = eta on clearance, η(v) = eta on volume of distribution

The estimated primary parameters are within the range of the few published models, clearance in the studies ranged from 2.81 to 4.8 L/hr and the volume of distribution from 68 to 130 L (49, 97-100) as detailed in table 5.3.

Table 5.3: previously published pretomanid population pharmacokinetic models and the estimated primary parameters

Publication	Year of publication	Ka (1/l)	clearance (l/hr)	volume (l)
Lyons MA	2022	0.82	3.4	100
Zou Y *et al*.	2022	0.396	2.1	68
Ignatius EH *et al*.	2021	0.592	3.91	90.9
Salinger DH *et al*.	2019	1.38	3.3	90.4
Lyons MA	2018	0.3	4.8	130

5.3.5. MIC

The distribution of MGIT MICs of pretomanid in pure isolates of M. tuberculosis from 478 TB-PRACTECAL study participants disaggregated by country of enrolment are presented in Figure 5.5. The median MIC was 0.125mg/L and the interquartile range from 0.125 to 0.25mg/L. 100% of the isolates were below the provisionally set critical concentration of 1mg/L (101) or 2mg/L (102). Only two isolates, both from SA study participants had a baseline MIC of 1.0mg/L.

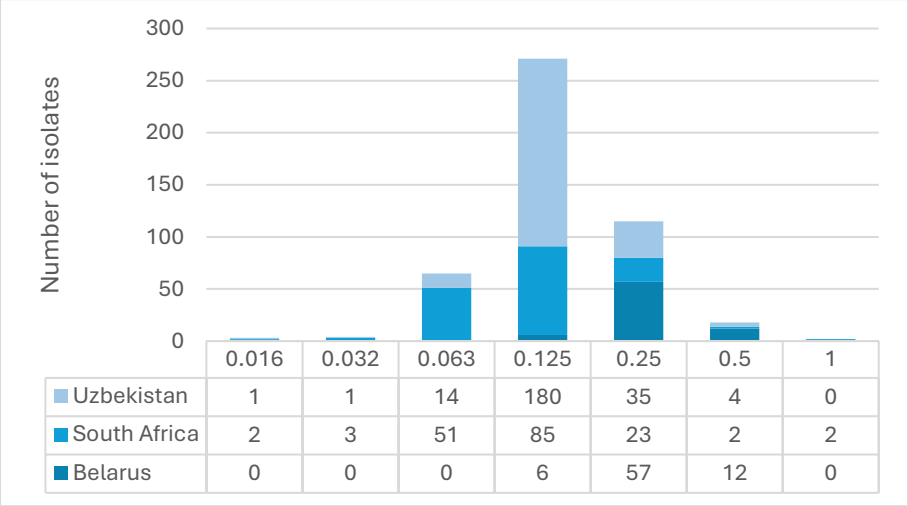


Figure 5.5: Distribution of M.tb baseline isolates across various pretomanid MICs in mg/L in the TB-PRACTECAL trial

5.3.6. Clinical simulations

Percentage Time above MIC

Simulating 2,000 virtual patients at the 200mg daily dose, the percentage time the free drug concentration was above MIC showed that only up to MIC of 0.25mg/L did the patients achieve the observed maximum effect (EC80) of 77% *f*T>MIC (Figure 5.6). Only at the lowest MIC of 0.016mg/L would all simulated doses achieve the maximal bactericidal effect at all assumed protein binding proportions. Our simulations indicated that there were no intermediate target attainments (22% or 48%), it was either 77% or nothing (figure 5.6 and appendix 8).

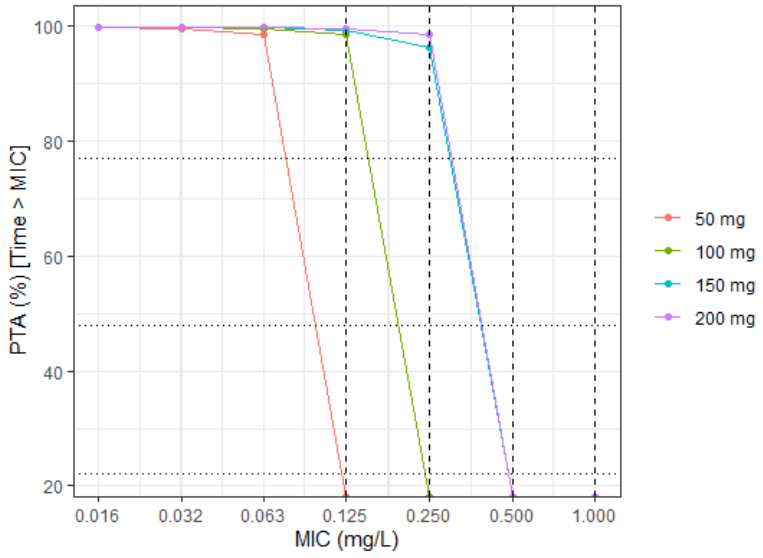


Figure 5.6: A plot of probability of fT%>MIC for the doses 50, 100, 150 and 200mg at assumed protein binding of 85%

AUC0-24/MIC

At an MIC of 0.0063mg/L and below, the 200mg dose had a higher than 90% probability of achieving the *f*AUC$_{0\text{-}24}$/MIC of 167 target when assumed protein binding was 85% (Figure 5.7).

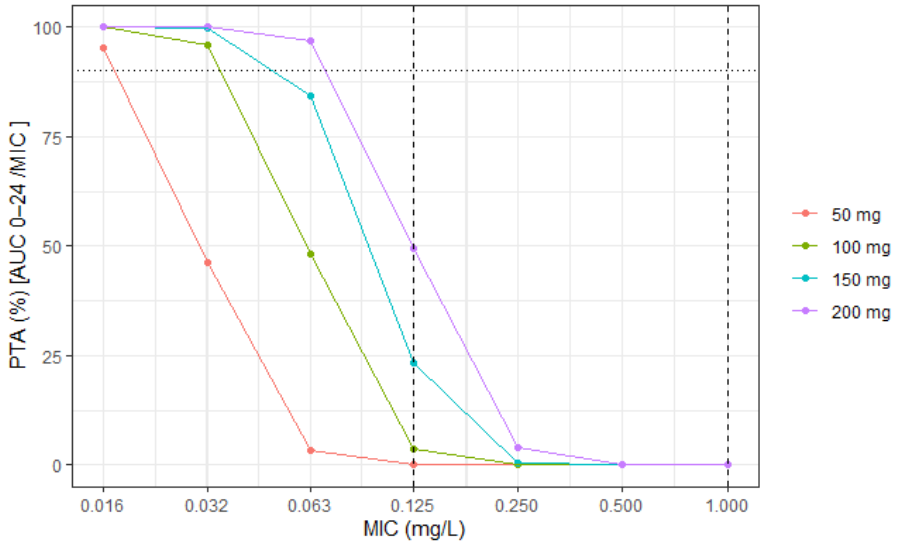


Figure 5.7: Plot of the probability of attaining the fAUC$_{0\text{-}24}$/MIC of 167 for 200mg, 150mg, 100mg and 50mg doses at an 85% protein binding assumption.

Varying assumptions in protein binding between 5% and 15% altered the overall conclusion on the *f*AUC$_{0\text{-}24}$/MIC PTA by at least 1 dilution per 5% increase in protein binding as shown in Figure 5.8. A table with PTA simulations for the other simulated doses is in supplementary appendix 7.

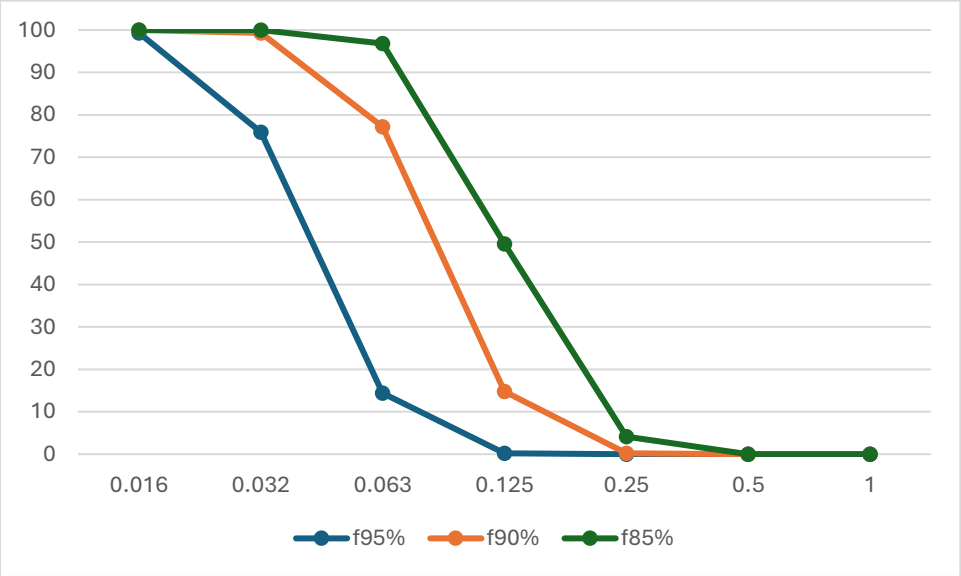


Figure 5.8: Probability of attaining an fAUC$_{0\text{-}24}$/MIC of 167 for a 200mg dose, varying protein binding assumptions.

5.4. Discussion

In regimens comprising bedaquiline, pretomanid and linezolid as a backbone, a one-compartment first order absorption and elimination model with allometric scaling of fat-free mass on both clearance and volume of distribution best characterised pretomanid pharmacokinetics. The pretomanid median MIC at baseline in the study population was 0.125 mg/L. Virtually all patients in the TB-PRACTECAL trial had drug exposures above %*f*T>MIC targets and at least 96% would have been above the *f*AUC/MIC target.

The clearance in our study was estimated at 3.08 L/hr, this is similar to clearances which have been previously published (49, 97-100). The only identified covariate that modified clearance in our study was fat-free mass estimated as 0.10 (Table 5.2 and Appendix 4 and 5), those identified from previous studies have also been body size related such as weight (49, 97) and FFM (99).

Food administration, particularly high calorie, high fat meal, is postulated to significantly increase pretomanid exposure (47, 99), however other studies have found the fed state to influence absorption but not bioavailability and consequently exposure (98). Although our study participants were encouraged to eat before taking medicines, there was no standardised meal, observation or recording of type of meal consumed and therefore current exposures are expected to be representative of real-world scenarios.

Co-administration of potent CYP450 inducers such as efavirenz, lopinavir/ritonavir and rifamycins (48, 99) reduces pretomanid exposure, however these drug classes were contraindicated in the TB-PRACTECAL trial. All the 39 participants living with HIV (42% of total study participants) were on integrase inhibitors and nucleotide reverse transcriptase inhibitor antiretroviral regimens, since HIV was not a significant covariate no further exploration of individual drugs' effect on the PK model was done. Both moxifloxacin and clofazimine are metabolised in the liver (103, 104), however including BPaLM, BPaLC and BPaL as covariates did not improve the model fit significantly (appendix 4), suggesting none or limited impact of the accompanying anti-TB drugs in the regimen on pretomanid exposure.

Study participants had a median baseline pretomanid MGIT MIC of 0.125mg/L, with 72% of them having mycobacteria that had an MIC of equal to or lower than 0.125 mg/L. Using Middlebrook 7H11, pretomanid MIC has been reported to range from 0.03125 to 0.25 mg/L (105) . At the standard 200mg daily dose that was used in the PRACTECAL study, assuming 85% protein binding, all isolates within this published range would have achieved the maximal efficacy %*f*T>MIC targets for pretomanid (figure 5.6). Although at a dosing interval of less than 72 hours as used in the PRACTECAL trial both AUC/MIC and T> MIC indices can be utilised (95), results from our study suggests the results would be interpreted differently. At the trial's dose of 200mg daily, adequate exposure would be achieved for strains with an MIC of 0.063 mg/L or below when using the *f*AUC/MIC 167 target; and strains with an MIC of 0.25 or lower when using the %*f*T>MIC above 77% target (figures 5.6 – 5.7). Further studies to establish the best pretomanid index in clinical PKPD, preferably utilising MGIT MIC are needed.

Some of the key strengths of this study is that it develops a pretomanid population pharmacokinetic model from the largest cohort of participants to date from a single study and includes participants from South Africa and Belarus; previous studies with dosing of up to six months were limited to one country. These results also confirm the adequate pretomanid exposure in rifampicin resistant TB regimens of BPaLM and BPaL which are the currently recommended regimens by WHO (32).

By not closely observing food intake, our study may under- or over-estimate the pretomanid drug exposure, although this has previously been shown not to be clinically significant. The trial excluded patients with moderate liver and renal function abnormality which makes it less representative of the full spectrum of RR-TB patients. Although the optimal design analyses indicated that the timing of the samples were adequate, the sampling was originally optimised for linezolid pharmacokinetics (79) and the sample size was smaller than modelled, however it was still possible to estimate Ka.

5.5. Conclusion

Pretomanid when given at 200mg daily in combination with bedaquiline, linezolid with/without moxifloxacin or clofazimine results in adequate exposure. This is the case also in HIV coinfected patients taking antiretroviral treatment consisting of integrase

inhibitors and nucleoside reverse transcriptase inhibitors. Assumed protein binding and type of PKPD index alter the interpretation of probability of target attainment significantly. Further studies to establish pretomanid protein binding in people, optimal PKPD PTA index and target are recommended.

5.6. Chapter 5 supplementary appendices

5.6.1. Appendix 1: Used r packages

```
library(rxode2)

library(nlmixr2)

library(reshape2)

library(ggplot2)

library(tidyverse)

library(PerformanceAnalytics)

library(psych)

library(dplyr)

library(GGally)
```

5.6.2. Appendix 2: R code for final model

```
ini({
        lka <- -1.15259732127294
label("Absorption rate")
lcl <- 1.13008124732802
label("Clearance")
lvc <- 4.62170931536226
label("Central volume of distribution")
prop.err <- c(0, 0.321731821549103)
add.err <- c(0, 367.831309683669)
covffmPow1 <- fix(0.75)
covffmPow2 <- fix(1)
eta.cl ~ 0.102674627530168
eta.vc ~ 0.10705836808838
 })

model({
ka <- exp(lka)
cl <- exp(lcl + eta.cl + logFFM * covffmPow1)
vc <- exp(lvc + eta.vc + logFFM * covffmPow2)
linCmt() ~ prop(prop.err) + add(add.err)
 })
```

5.6.3. Appendix 3: Table summarising base model selection

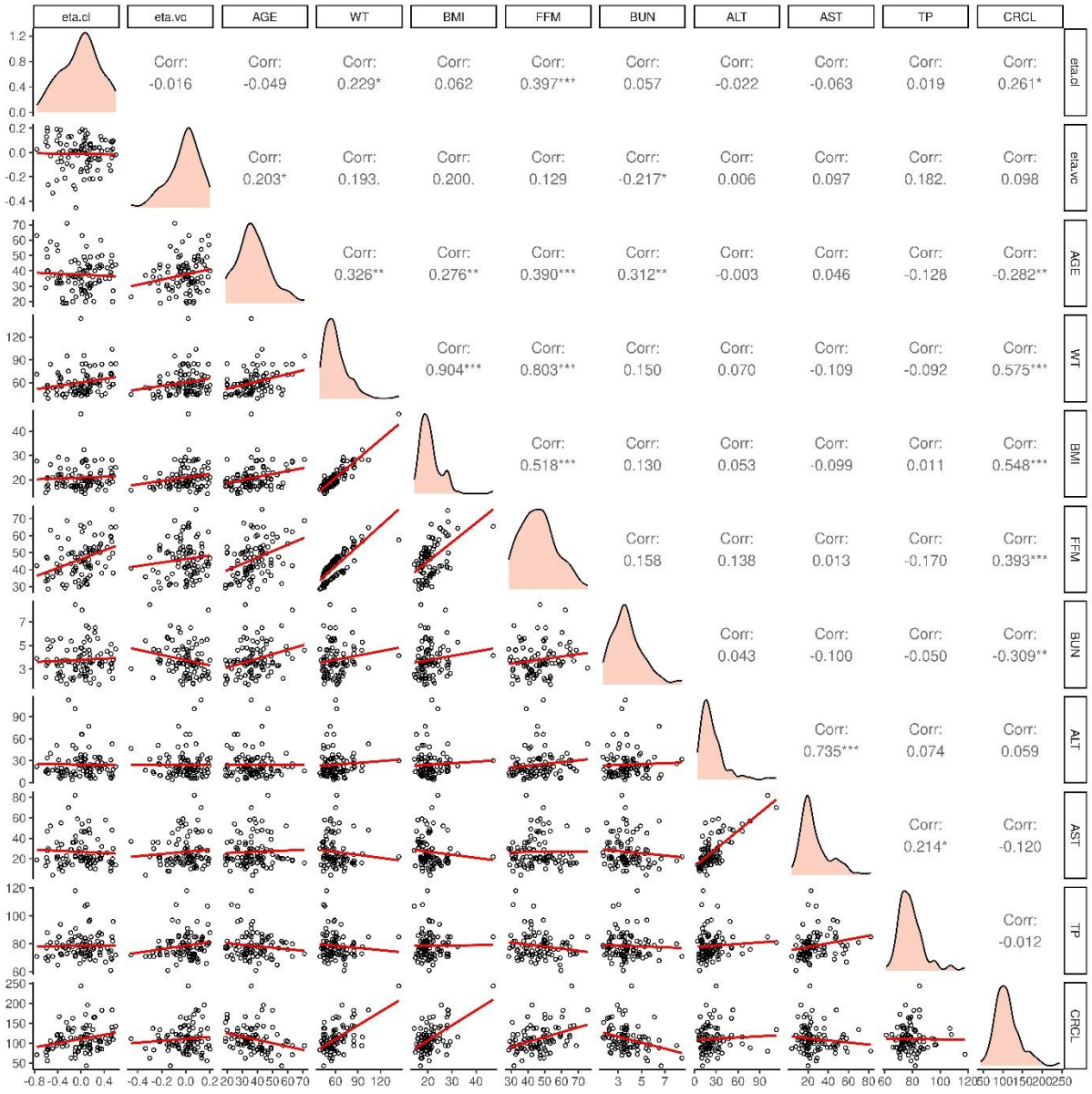
Base model selection

Run No	Model Description	Reference model	AIC	OFV	ΔAIC	ΔOFV	Comments
1	1CMPT, ETA $[CL, V_c]$, Combined RV	Run 1	13250.71	11738.84	0.00	0.00	**Base Model**
2	2CMPT, ETA $[CL, V_c]$, Combined RV	Run 1	13249.04	11733.17	-1.67	-5.67	High RSE of Q
3	Change to proportional RV	Run 1	13957664.07	13956154.20	13944413.36	13944415.36	Failed covariance
4	Change to additive RV	Run 1	13525.89	12016.02	275.18	277.18	No decrease in OFV/AIC
5	Change to log-normal additive RV	Run 1	14487.38	12977.51	1236.67	1238.67	No decrease in OFV/AIC
6	Add transit compartment	Run 1	13251.07	11739.20	0.36	0.36	No decrease in OFV/AIC
7	Estimate ETA for Ka	Run 1	13256.44	11742.57	5.73	3.73	High shrinkage of Ka
8	Introduce correlation between IIV on CL and Vc	Run 1	13251.68	11737.81	0.97	-1.03	No decrease in OFV/AIC

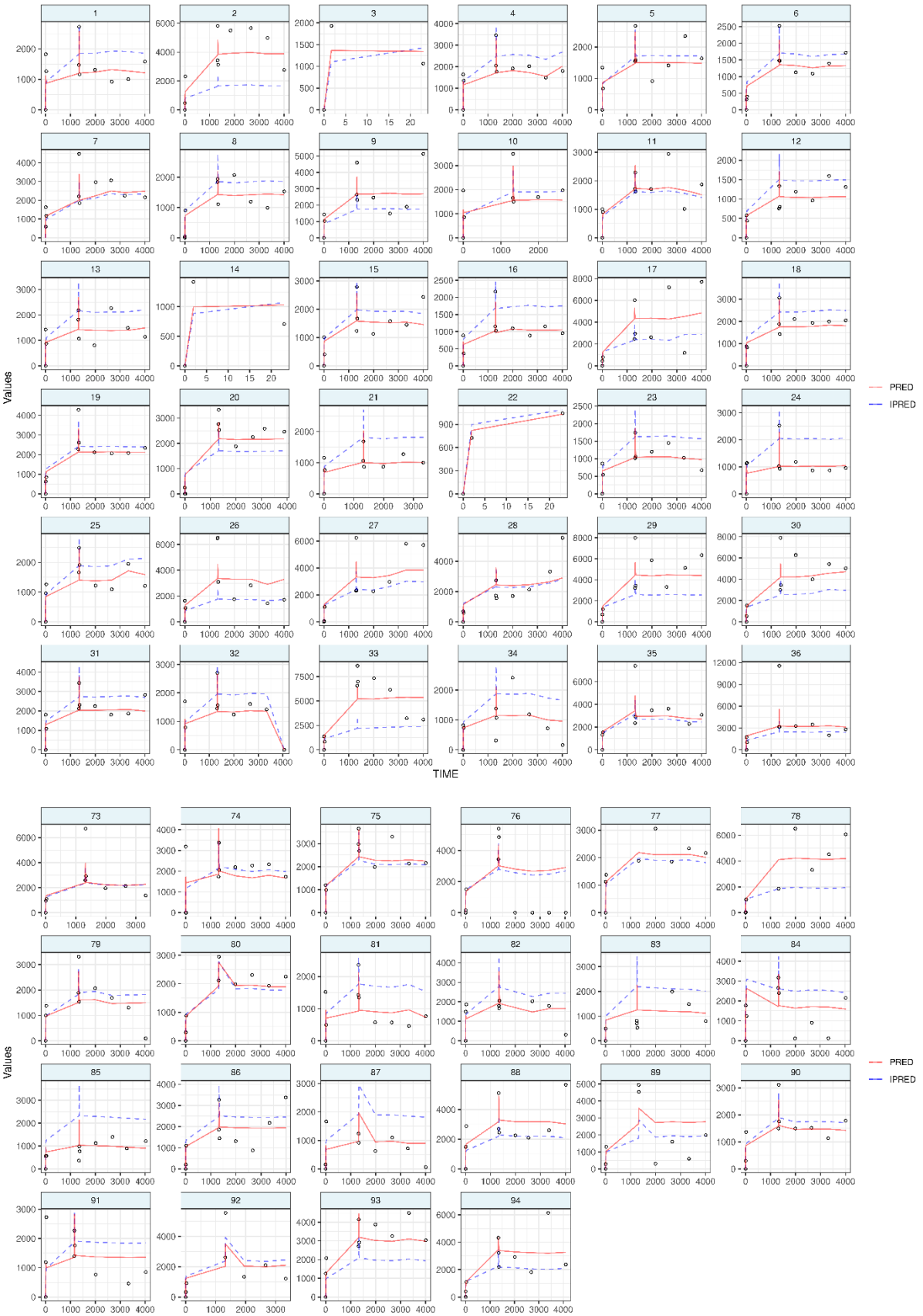
5.6.4. Appendix 4: Table summarising covariate model selection

Run No	Model Description	Reference model	OFV	ΔOFV	Significant (Y/N)
1	Base model	-	11690.74	-	-
	Round 1				
2	Introduce allometric scalling (WT)	Run 1	11680.71	-10.03	Y
3	Introduce allometric scalling (BMI)	Run 1	11690.06	-0.68	N
4	**Introduce allometric scalling (FFM) [Final Model]**	**Run 1**	**11673.23**	**-17.51**	**Y**
	Round 2				
5	Introduce AGE on CL	Run 4	11670.15	-3.08	N
6	Introduce BUN on CL	Run 4	11673.34	0.11	N
7	Introduce ALT on CL	Run 4	11672.16	-1.07	N
8	Introduce AST on CL	Run 4	11673.14	-0.09	N
9	Introduce AST on CRCL	Run 4	11671.45	-1.78	N
10	Introduce TP on CL	Run 4	11673.73	0.5	N
11	Introduce FEMALE on CL	Run 4	11672.75	-0.48	N
12	Introduce CAUCASIAN on CL	Run 4	11672.98	-0.25	N
13	Introduce BLACK on CL	Run 4	11672.93	-0.3	N
14	Introduce HIV on CL	Run 4	11672.83	-0.4	N
15	Introduce TREATMENT1 on CL	Run 4	11673.74	0.51	N
16	Introduce TREATMENT2 on CL	Run 4	11671.31	-1.92	N
17	Introduce TREATMENT3 on CL	Run 4	11672.48	-0.75	N
18	Introduce AGE on Vc	Run 4	11673.83	0.6	N
19	Introduce TP on Vc	Run 4	11674.13	0.9	N
20	Introduce FEMALE on Vc	Run 4	11665.17	-8.06	Y
21	Introduce CAUCASIAN on Vc	Run 4	11668.85	-4.38	Y
22	Introduce BLACK on Vc	Run 4	11667.92	-5.31	Y
23	Introduce HIV on Vc	Run 4	11667.61	-5.62	Y
24	Introduce TREATMENT1 on Vc	Run 4	11672.53	-0.7	N
25	Introduce TREATMENT2 on Vc	Run 4	11670.79	-2.44	N
26	Introduce TREATMENT3 on Vc	Run 4	11666.46	-6.77	Y
	Round 3				
27	Introduce CAUCASIAN on Vc	Run 20	11660.16	-5.01	Y
28	Introduce BLACK on Vc	Run 20	11658.7	-6.47	Y
29	Introduce HIV on Vc	Run 20	11660.09	-5.08	Y
30	Introduce TREATMENT3 on Vc	Run 20	11659.23	-5.94	Y
	Round 4				
31	Introduce CAUCASIAN on Vc	Run 28	11658.83	0.13	N
32	Introduce HIV on Vc	Run 28	11657.82	-0.88	N
33	Introduce TREATMENT3 on Vc	Run 28	11652.85	-5.85	Y
	Stepwsie backforward elimination				
34	Remove FFM from CL, Vc	Run 33	11679.86	27.01	Y
35	Remove FEMALE from Vc	Run 33	11661.12	8.27	N
36	Remove TREATMENT3 from Vc	Run 33	11658.7	5.85	N
37	Remove BLACK from Vc	Run 33	11659.23	6.38	N

5.6.5. Appendix 5: Continuous variates matrix plot – against etas



5.6.6. Appendix 6: Individual patient pretomanid concentration plots

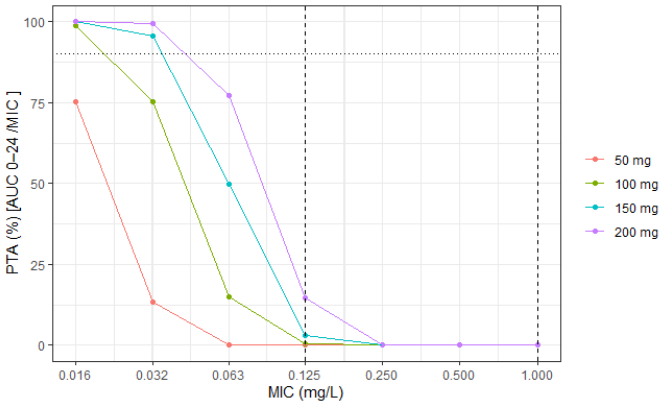

Values
TIME
PRED
IPRED

5.6.7. Appendix 7: fAUC$_{0\text{-}24}$/MIC of 167 PTA for 200mg, 150mg, 100 and 50mg doses with varying protein binding assumptions

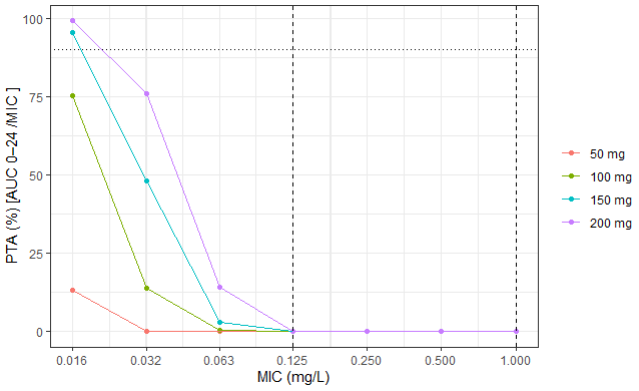
Table of probability of achieving an AUC$_{0\text{-}24}$/MIC of 167 by dose at varying protein binding proportions

		MIC mg/L																				
		0.016			0.032			0.063			0.125			0.25			0.5			1		
	protein binding	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
AUC/MIC target achievement	200mg	99.3	100	100	75.9	99.3	100	14.35	77.2	96.75	0.25	14.75	49.55	0	0.25	4.1	0	0	0	0	0	0
	150mg	95.5	98.95	100	48.05	95.5	99.8	3.1	49.85	84.25	0	3.2	23.3	0	0	0.35	0	0	0	0	0	0
	100mg	75.35	98.95	99.95	14	75.35	95.95	0.35	15.05	48.1	0	0.35	3.8	0	0	0.05	0	0	0	0	0	0
	50mg	13.35	75.3	95.3	0.1	13.35	46.4	0	0.15	3.5	0	0	0	0	0	0	0	0	0	0	0	0

Red is below 90% PTA



Plot of probability of achieving an AUC$_{0\text{-}24}$/MIC of 167 by dose at an assumed 90% protein binding

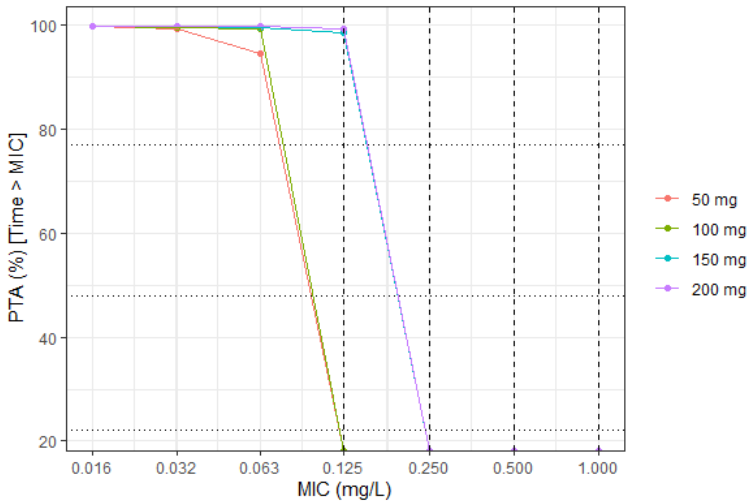


Plot of probability of achieving an AUC$_{0\text{-}24}$/MIC of 167 by dose at an assumed 95% protein binding

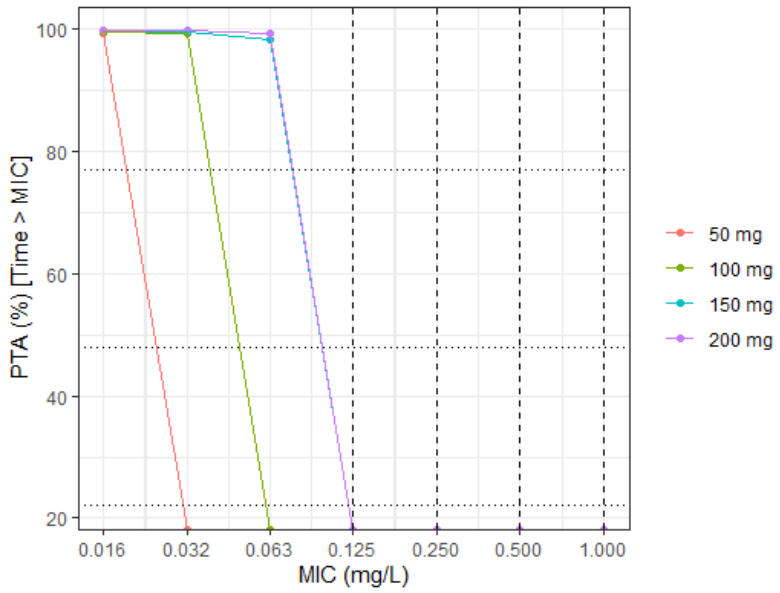
5.6.8. Appendix 8: fT>MIC PTA for 200mg, 150mg, 100 and 50mg doses with varying protein binding assumptions

Table of %*f*T>MIC by dose and free drug %

		0.016			0.032			0.063			0.125			0.25			0.5			1		
	protein binding	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
%T>MIC target achieveme	200mg	99.78	99.78	99.78	99.74	99.78	99.78	99.37	99.75	99.78	0	99.38	99.67	0	0	98.71	0	0	0	0	0	0
	150mg	99.77	99.78	99.78	99.67	99.77	99.78	98.48	99.67	99.76	0	98.51	99.48	0	0	96.41	0	0	0	0	0	0
	100mg	99.73	99.78	99.78	99.32	99.73	99.76	0	99.34	99.67	0	0	98.62	0	0	0	0	0	0	0	0	0
	50mg	99.37	99.76	99.77	0	99.37	99.7	0	94.66	98.64	0	0	0	0	0	0	0	0	0	0	0	0



Plot of %*f*T>MIC by dose at an assumed 90% protein binding



Plot of %*f*T>MIC by dose at an assumed 95% protein binding

Chapter 6: Clofazimine population pharmacokinetics and probability target attainment in adults on BPaL based treatment for rifampicin-resistant tuberculosis.

6.1. Introduction

This chapter reports on the study methods and results of the population pharmacokinetics of clofazimine and its probability target attainment. The results section describes the participants of the study, the pharmacokinetic data that was used and the population pharmacokinetic model building. The intermediate model building steps detail out the structural, statistical and covariate model building and evaluation of the final linezolid population pharmacokinetic model. The parameter estimates derived from the final model are presented, including a discussion on how they compare with those from previously published papers. Clofazimine MICs in the TB-PRACTECAL clinical trial (72) are presented and used in the probability target attainment analyses. Finally, the role of these results in informing the use and dosing of clofazimine in the treatment of tuberculosis is discussed.

6.2. Methods

6.2.1. Study design

This was a sub study nested in a randomised controlled trial in patients with rifampicin resistant tuberculosis. Participants randomised to arm two of the trial received bedaquiline 400mg daily for 2 weeks then 200mg three times a week for 22 weeks, pretomanid 200mg daily for 24 weeks and tapered dose linezolid 600mg daily for 16 weeks then 300mg for 8 weeks and clofazimine 100mg daily for 24 weeks. PK blood samples were collected on Day 1 (0, 2 and 23 hours), Weeks 8 (pre-dose, 6.5 and 23 hours), 12, 16, 20, 24, 32 and 72 post randomisation visits. Drug concentrations were quantified in a GCP laboratory using a high-performance liquid chromatography-tandem mass spectrometry. The lower limit of quantification for clofazimine was 7ng/mL.

6.2.2. Pharmacometric analysis

nlmixr2, an open-source R package was used for population PK modelling and simulation estimation. R v4.1.2 was used for dataset creation, data exploration and generation of tables and plots. The list of the r packages used is in appendix 1. The PopPK for clofazimine was analysed using a non-linear mixed effect modelling approach. The first-order conditional estimation with interaction (FOCE-I) algorithm in nlmixr2 was used. Inter-individual variability (IIV) at the parameter level and residual variability (RV) at the observation level made up the mixed effects analysis.

6.2.3. Structural model

The PopPK study first explored basic model structure based on the observed plasma concentration data. One-, two-, three- and four-compartment linear models were evaluated respectively with combined, proportional, additive and log-transformed residual error models. Finally, random effects on clearance (CL) and volume of distribution (V) without correlation were included in the model. Fixed lag times and fixed and estimated absorption rate constant (ka) absorption models were explored.

6.2.4. Covariate model

A covariate matrix of continuous variables including age, weight, BMI, FFM, BUN, ALT, AST, TP, CLCR and eta estimates on clearance and volume of distribution from the base model explored correlation as well as covariate collinearity. Association between categorical covariates (sex, HIV status, race) and etas on clearance and volume were also independently explored. Allometric scaling was applied to both volume of distribution and clearance. The coefficients of the power model were fixed to 1 for V and 0.75 for CL. The selected covariates underwent stepwise forward inclusion ($P<0.05$, $\Delta OFV > 3.84$) and backward elimination ($p<0.001$, $\Delta OFV > 10.83$) to select those that would improve the model fit significantly.

6.2.5. Model evaluation

Goodness-of-fit plots were used to assess how well the model predicted individual and population values closely matched the observed PK data. Model validation was also performed using visual predictive check (VPC) plots and non-parametric bootstrap. The shrinkage, relative standard error, and variability value including omega and sigma value were also used to assess the precision and robustness of the model.

6.2.6. MIC

Minimum inhibitory concentrations were determined from a routine testing concentration set (2, 1, 0.5, 0.25, 0.125, 0.063 mg/L) in MGIT; testing was performed using a lower (0.032 mg/L) testing concentration if required. The results from all participants from the TB-PRACTECAL trial were summarised by country of enrolment and the median and interquartile range reported.

6.2.7. Probability of Target Attainment

Despite in vitro promise (106) and hypothesised added value in shortening of treatment regimens (107), demonstrating the direct efficacy of clofazimine in patients with TB has proven elusive (37) and consequently an effective dose and PKPD target have not yet been established. The duration of serum drug concentration above the MIC has been associated with clofazimine's sustained antimicrobial activity. We therefore used the percentage above the MIC (%T>MIC) as the PKPD index (108). Clofazimine is highly protein bound, in vitro studies have demonstrated the free drug to be less than 15% (109) while others have even suggested it to be lower than 1% (110).

Using 2,000 Montecarlo simulations, the clofazimine %T>MIC at week 24 of treatment was calculated for the MIC range observed in the TB-PRACTECAL trial (0.063 – 2 mg/L) and T(days)>MIC during the 12-month post treatment period.

6.3. Results

6.3.1. Study population

30 study participants (23% female) with a median age of 35 years (range: 19 – 50 years) (see table 6.1) contributed 286 timed plasma samples which upon bioanalysis were included in the clofazimine PopPK dataset. 23 samples were collected before the first dose and 263 samples after the first dose. 38 samples were deemed to be below the

Table 6.1: baseline characteristics of study participants (n = 30)

Characteristic	Total
Female, n (%)	7 (23)
Age, years (range)	35 (19-50)
Race, n (%)	
Asian	1 (3)
Black	17 (57)
Caucasian	12 (40)
HIV status, n (%)	
positive	10 (33)
negative	20 (67)
Weight, kg	56.8 (39.2 – 104)
Height, cm	172 (152 – 192)
BMI, Kg/m2	19.3 (14.3 – 28.2)
Fat Free Mass, kg	47.1 (28.6 – 75.5)
BUN (mmol/L)	3.5 (1.8 – 8.5)
ALT (IU/L)	19 (4 – 77)
AST (IU/L)	22 (10 – 58)
ALP* (IU/L)	63 (36 – 102)
Albumin* (g/L)	44 (36 – 49)
Total protein (g/L)	75 (67 – 107)
Creatinine (mcrmol/L)	63 (37 – 111)
Creatinine clearance (mL/min)	107.9 (62.8 – 183.5)

Median (min-max) if not stated otherwise. * n=12

BMI= body mass index, ALT= alanine transaminase, AST= aspartate aminotransferase, ALP=alkaline phosphatase, BUN= blood urea nitrogen

The observed concentrations of clofazimine ranged from 10.78 – 1467.85 ng/ml. The median trough concentration was 365.40 ng/ml, with an interquartile range of 102.51 – 528.55 ng/ml (see Figure 6.1).

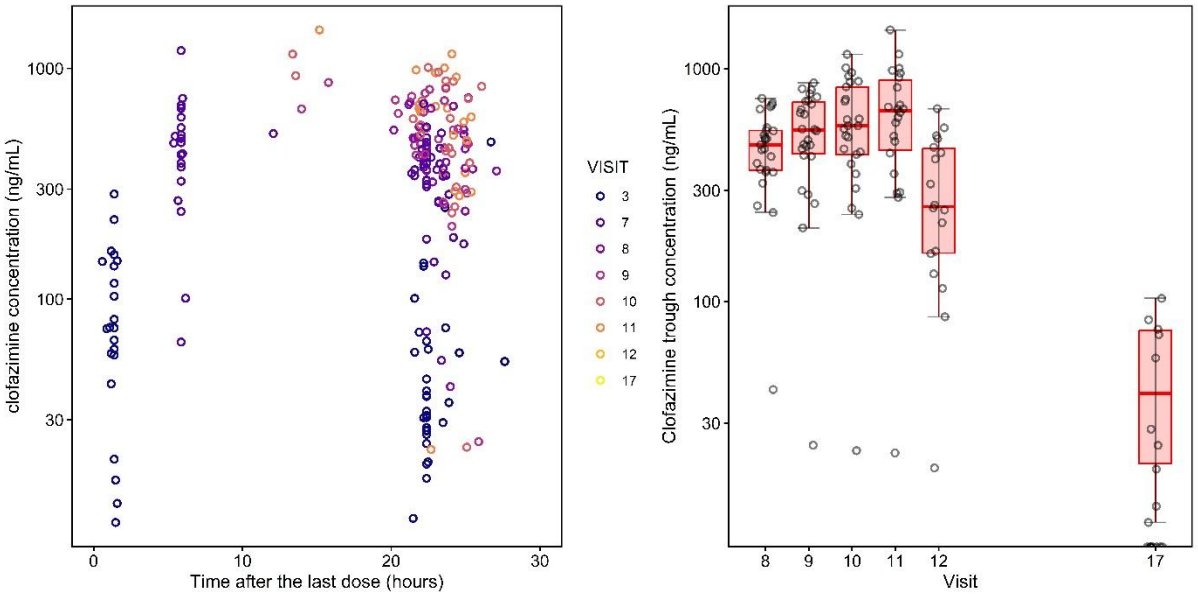


Figure 6.1: Plots of observed clofazimine concentrations by time after last dose on the left. Trough (visits 8-11 [weeks 12-24]) and post treatment completion (visits 12 and 17 [weeks 32 and 72]) concentrations by time after first dose aggregated by study visit number (right). The pink box represents the interquartile range with the red line representing the median while the whiskers represent the 5th and 95th percentiles.

The observed pk plots for each individual patient are shown in supplementary appendix 6.

6.3.2. Structural and variability model

A two-compartment first order absorption and elimination model with an OFV of 2783.52 with a fixed lag time and absorption constant from a previous publication (111) was selected as the best to characterise the clofazimine observed PK data. Random effects on clearance, central volume of distribution, peripheral volume of distribution and inter-compartmental model were included in the model to explain the inter-individual variability. Combined residual error model was used for the unexplained variability as it performed better than additive (OFV = 2900.81), proportional (OFV = 3608.12) and log-transformed additive (OFV = 4582.03) models. The structural model is represented by figure 6.2 and the summary of model evaluation results with OFVs and delta ΔOFVs is in supplementary appendix 3 [models run 1-19]

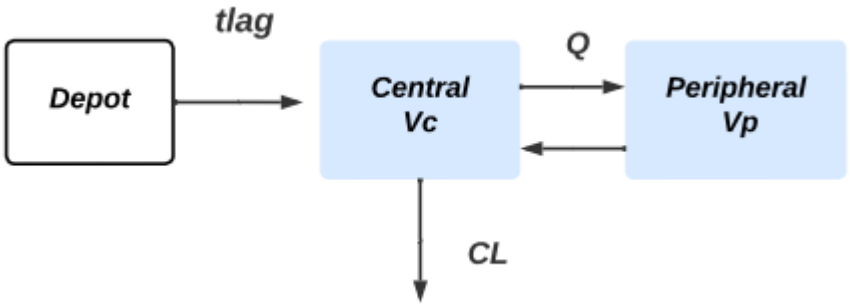


Figure 6.2: Schematic representation of the structural clofazimine model

tlag = lag time, Vc = central compartment volume, CL = clearance, Q = intercompartmental clearance, Vp = peripheral compartment volume

The base model's r code is in appendix 2 and the ordinary differential equations are as follows [Equation 6.1]:

$$\frac{dA_{depot}}{dt} = -K_a \times A_{depot}(t)$$

$$\frac{dA_{central}}{dt} = K_a \times A_{depot} - \frac{CL}{V_c} \times A_{central}(t) - Q/V_c * A_{central} + Q/V_p * A_{peripheral1}(t)$$

$$\frac{dA_{peripheral}}{dt} = Q/V_c * A_{central} - Q/V_p * A_{peripheral}(t)$$

where A_{depot} is the amount of clofazimine in the depot compartment, $A_{central}$ is the amount of clofazimine in the central compartment, K_a is the absorption rate constant for the transfer of clofazimine from second transit compartment to central compartment, CL/F is the apparent clearance of clofazimine, V_c/F and V_p is the apparent volume of distribution of clofazimine for central and peripheral compartment. Q is the inter-compartmental clearance.

6.3.3. Covariate model

No covariate with significant impact was observed during covariate selection. Body weight allometric scaling was integrated and the parameters of the final model were scaled to represent a person with a weight of 70 kg. A matrix plot of chosen model's covariates corelations in appendix 4.

6.3.4. Final model evaluation

Goodness-of-fit plots for the final PopPK model showed no significant bias from the unity line in both PRED VS DV and IPRED VS DV, indicating that the model predicted individual and population values closely matched the observed PK data (Figure 6.3).

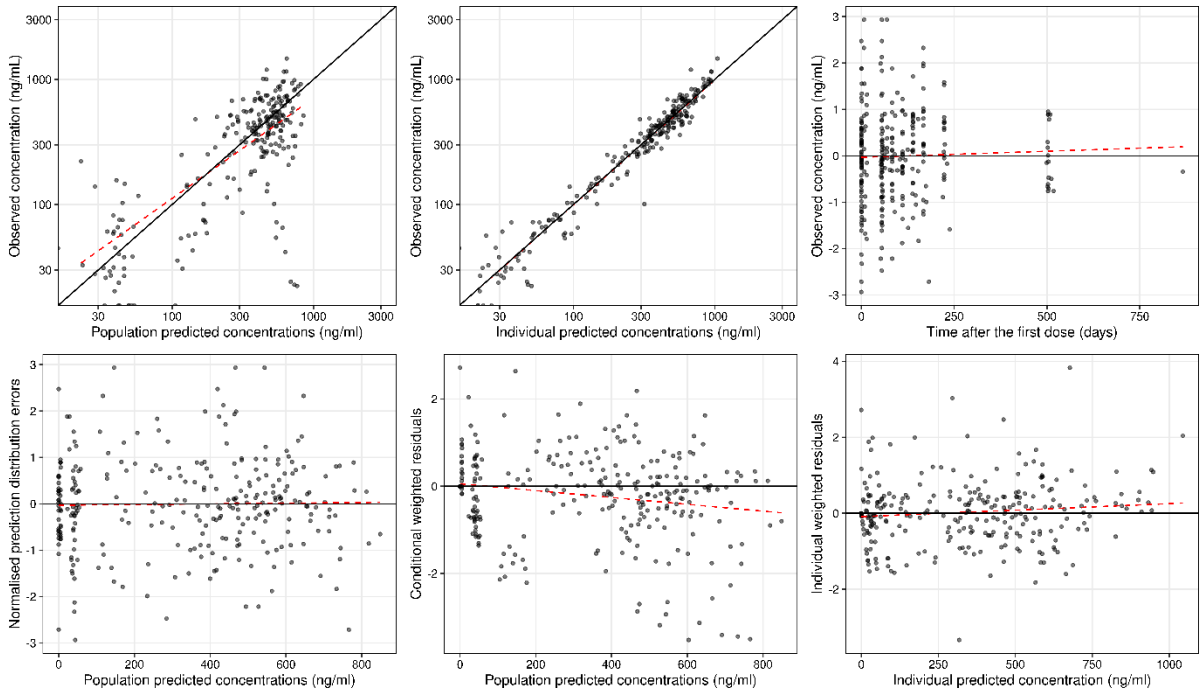


Figure 6.3: Final clofazimine model goodness of fit plots clockwise from top left: DV vs PRED, DV vs IPRED, DV vs TAFD, IWRES vs IPRED, CWRES vs PRED, NPDE vs PRED

Model validation using a visual predictive check (VPC) plot visually showed the predictive accuracy of the final model (figure 6.4).

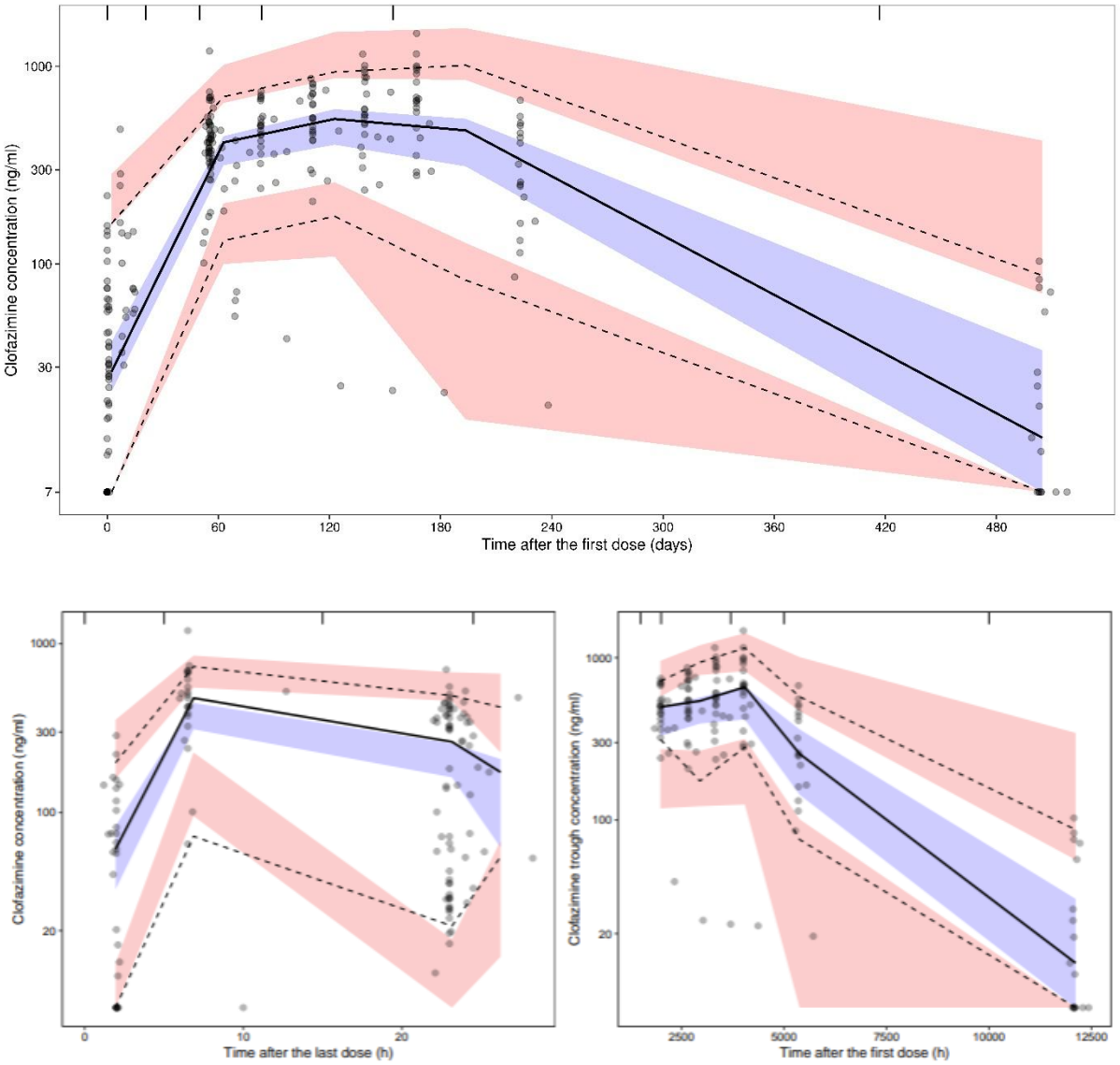


Figure 6.4: Final clofazimine model VPC plots. The black circles in the figure represents the observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. purple area represents a simulation-based 95 % confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink shading.

The final model parameters are shown in table 6.2.

Table 6.2: Final clofazimine model parameters and bootstrap results

Parameters	Estimate (%RSE [a])	95% CI [b]	Bootstrap Median [95% CI [c]]
$K_a(h^{-1})^d$	0.67 (Fixed)	-	
$Tlag(h)$	0.62 (Fixed)	-	-
$CL/F(L/h/70kg$ [e])	6.84 (7.49)	5.16 - 9.06	6.91 [5.83 - 8.67]
$V_c/F(L/70kg)$	1750 (3.43)	1060 - 2890	1800 [1640 - 2430]
$V_p/F(L/70kg)$	9150 (1.17)	7420 - 11300	9200 [7730 - 11000]
$Q(L/h/70kg)$	41.7 (5.69)	27.5 - 63.3	42.3 [37.9 - 56.4]
Between subject variability [f]	Estimate	Shrinkage (%)	Bootstrap Median [95% CI]
IIV$CL(\%)$	77.3	8.78	74.6 [38.1 - 125]
IIV$V_c(\%)$	173	2.39	173 [169 - 191]
IIV$V_p(\%)$	44.6	26.1	44.5 [27.7 - 62.3]
IIV$Q(\%)$	82	40.9	81.5 [35.7 - 122]
Residual Error (σ):			
Proportional	0.198	-	0.195 [0.146 - 0.246]
Additive (mg/L)	0.0164	-	0.0164 [0.016 - 0.0165]

Abbreviations: K_a=absorption rate constant; Tlag= lag time of absorption; CL=clearance; Vc=central volume of distribution; Vp=volume of distribution of peripheral compartment; Q=inter-compartmental clearance

[a] RSE = relative standard error, calculated as 100 × (standard error (SE)/typical value).

[b] 95 % CI = 95 % percentile confidence interval, calculated as point estimate +/- 2 × SE

[c] In the bootstrap, 95 % CI was computed using 2.5th, and 97.5th percentiles of the parameter estimates from a bootstrap with 1000 samples.

[d] The final model incorporates an absorption model with lag time from published Paper [1], using fixed population estimates.

[e] Parameters were standardised to a 70-kg person with the power of 0.75 for clearance and inter-compartmental clearance, 1 for volume of distribution.

[f] BSV % reported in CV scale was calculated as $\sqrt{(e^{\omega^2}-1)}$ where ω is standard deviation for variability

The estimated primary parameters are within the range of the published models. Clearance in the studies using two-compartment models ranged from 3.71 to 12.5 L/hr and the combined volume of distribution from 3907 L to 9,200 L as detailed in table 6.3 below.

The terminal elimination half-life was 45 days, the alpha and beta half-life estimation tables are in supplementary appendix 6.

Table 6.3: previously published clofazimine population pharmacokinetic models and primary parameters

Publication	Year of publication	Structural model	clearance (l/hr)	volume (l)
Zhang CX *et al*(112).	2024	2 compartments	3.71	Vc 473, Vp 3434
Ali AM *et al.*(113)	2024	1 compartment	4.74	3200
Abdelwahab MT *et al.*(114)	2020	3 compartments	11.5	Vc 262, Vp1 10500, Vp2 889
Faraj A *et al.*(111)	2020	2 compartments	12.5	Vc 1138, Vp 8062
Strydom N *et al.*(87)	2019	1 compartment	16.3	280
Nix DE *et al.*(57)	2004	1 compartment	76.7	1470

6.3.5. MIC

The distribution of MICs of clofazimine in pure isolates of M. tuberculosis from 406 TB-PRACTECAL study participants disaggregated by country of enrolment are presented in Figure 6.5. The mode MIC was 0.125mg/L and the interquartile range from 0.125 to 0.25mg/L. 99.8% of the isolates were below the interim critical concentration of 1mg/L (115).

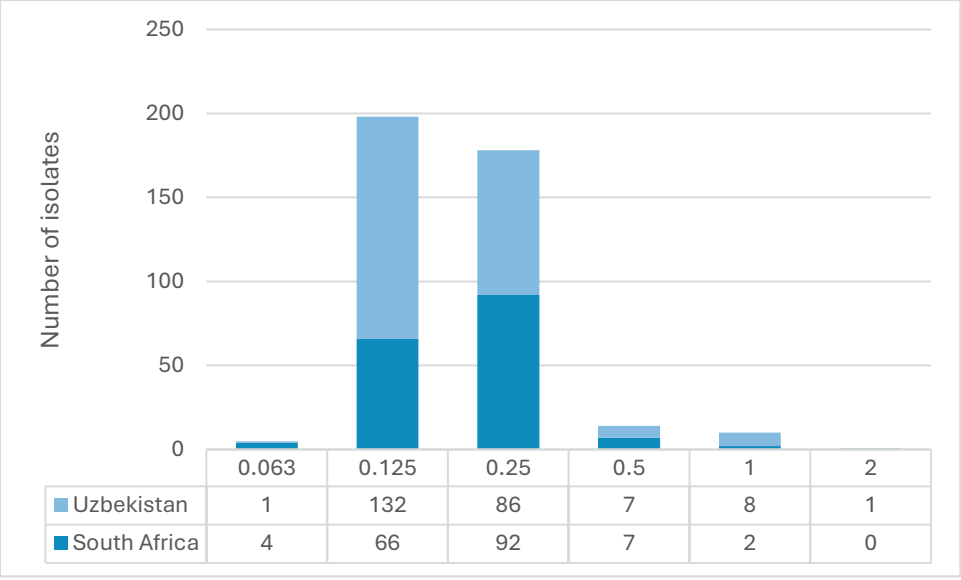


Figure 6.5: Distribution of M.tb baseline isolates across various clofazimine MICs (mg/L) in the TB-PRACTECAL trial

6.3.6. *Clinical trial simulations*

%T > MIC during treatment

We simulated the flat daily doses of 50mg, 100mg and 200mg, and loading dose of 200mg daily for eight weeks followed by 100mg daily and estimated the probability of target attainment of T>MIC of 100% at week 24 with the results presented in figure 6.6 and appendix 7.

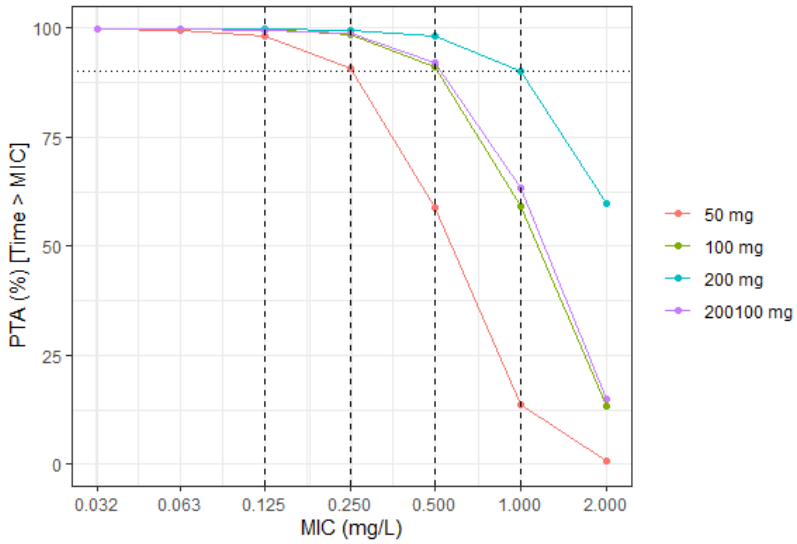


Figure 6.6: % T above MIC for four dosing approaches.

T (days) > MIC post treatment

At a dose of 200mg daily, clofazimine plasma concentration is maintained above 0.5mg/L for just under 50 days but may remain above 0.032mg/L beyond 12 months.

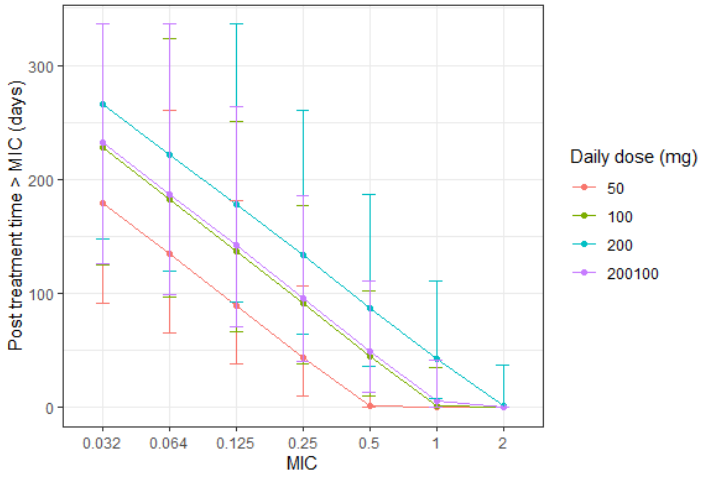


Figure 6.7: Time above MIC post-treatment completion

6.4. Discussion

A two-compartment first order absorption and elimination model with a lag time absorption parameter best described the pharmacokinetics of clofazimine in adults with rifampicin resistant tuberculosis from South Africa and Belarus. No covariate was identified so only allometric scaling on weight was integrated. Model evaluation using VPC (figure 6.4) and Bootstrap (table 6.2) confirmed the stability and precision of the final model. Using the 100mg daily flat dosing, the probability of the concentration remaining above the minimum inhibitory concentration was above 90% up to 0.5mg/L (figure 6.6).

The final model-predicted clearance of 6.84 L/hr is lower than the clearances reported in the adult TB patients' studies (111, 114), however the range of published clofazimine clearance is as slow as 3.71 L/hr (112) to as high as 76.6 L/hr (57). Although intense data was collected to accurately model the absorption phases in the Faraj *et al*. and Abdelwahab *et al*. papers (111, 114), the elimination phase was modelled based on samples collected up to 14 days from the last dose, in our study we included data from 2 months and 12 months post final dose, capturing the terminal elimination phase much closer. The large volume of distribution (Vc 1,750L and Vp 9,150L) is similar to sizes reported in the other 2-3 compartment models (111, 114). Only one study has reported a non-body size related covariate (114), sex, and they linked this to differences in body fat proportion.

The exposure from the PRACTECAL dose of 100mg daily and from the loading dose approach was similar as shown in figure 6.6. 92% of PRACTECAL participants had M.tb isolates with an MIC which would have resulted in 100% T>MIC. Only a 200mg daily dose simulation reached the target at the 1mg/L clofazimine critical concentration. Clofazimine protein binding is so high that when free drug assumptions are used in the PTA analysis, even at an MIC of 0.032 mg/L the clofazimine concentration is almost always below the MIC (appendix 7).

Clofazimine elimination half-life has been reported as 10.5 days (116), 34.2 days (114) and 70 days (56) with no established explanation to the variability. We report the terminal elimination half-life in our study as 45 days, having captured data points in the elimination phase up to 12 months after the last dose. The clofazimine long tail has been

postulated to be of value in treatment shortening by continuing to be effective long after treatment intake has ceased (108). Clofazimine has significant activity in bacillary persister populations (111), it also has higher lung and fibrous lesion concentration of up to 22 times the plasma concentration (87). Therefore, clofazimine could have a significant role post treatment to prevent recurrences as well as to protect against resistance development in other anti-TB drugs with a long half-life such as bedaquiline if recurrences occurred. This may explain the lower recurrences and absence of acquired bedaquiline resistant strains in the BPaLC regimen in comparison to the BPaL regimen (72). Although QT prolongation and consequently risk of Torsades du pointes have been raised as concerns when combining bedaquiline and clofazimine, in the TB-PRACTECAL trial, of 3,744 ECGs recorded over the 24 week treatment period for the investigational regimens, only one had a QTcF greater than 500ms (117).

The covariate models' likely convergence in local minima is a limitation, however there was no correlation between the etas and explored covariates as well and only a body proportion related covariate has been reported in one study in literature. The study had low sample size due to it being a sub-study and although the optimal design analyses indicated that the timing of the samples were adequate, the sampling was originally optimised for linezolid pharmacokinetics (79).

6.5. Conclusion

We present a clofazimine population pharmacokinetic model developed from the longest post treatment follow-up data reported to date. The long follow up period adequately captures and confirms the long terminal elimination half-life of 45 days. Reliable PKPD targets which take the high lipophilicity of clofazimine and its niche efficacy on persisters need further research.

6.6. Chapter 6 supplementary appendices

6.6.1. Appendix 1: Used r packages

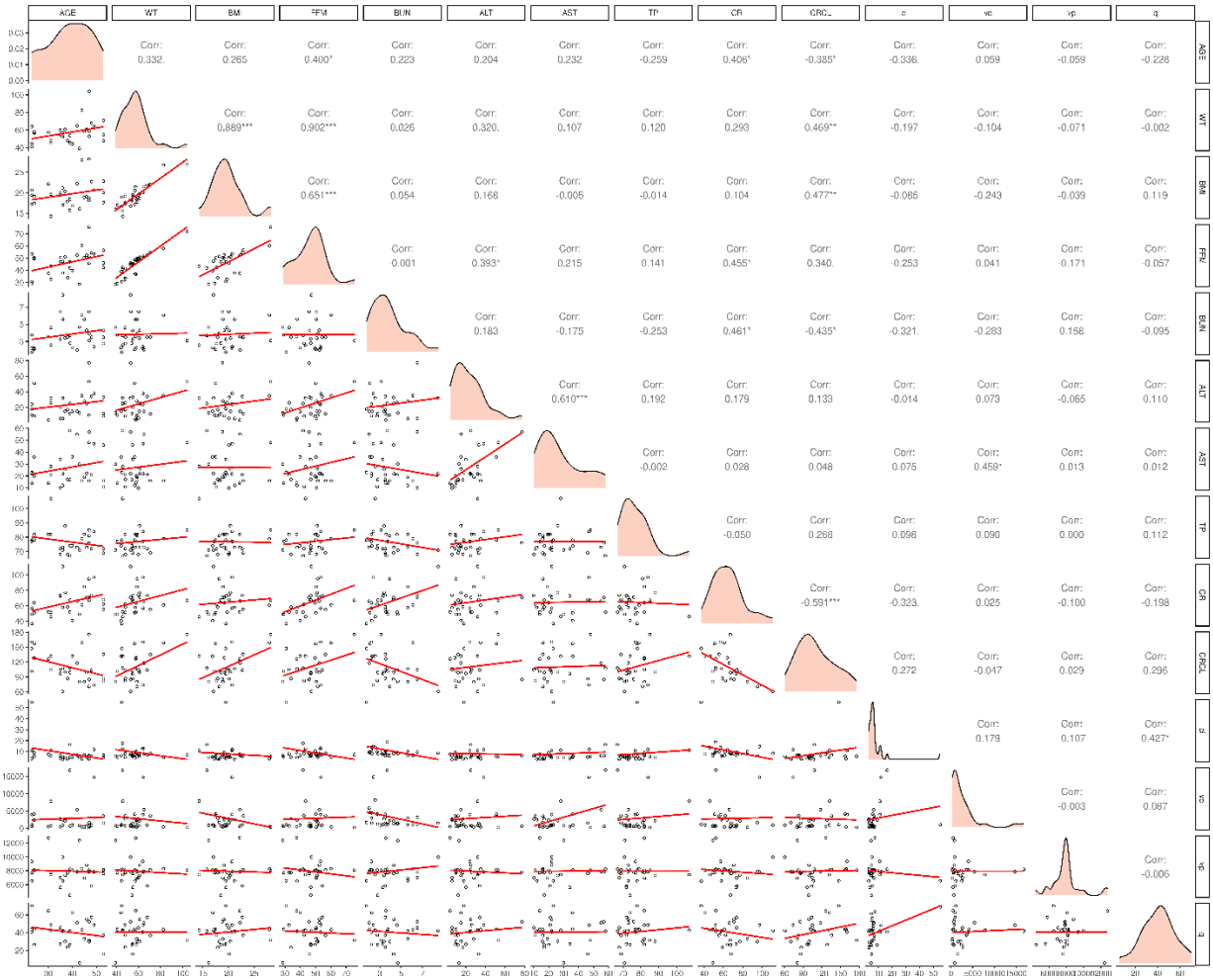
```
library(rxode2)

library(nlmixr2)

library(reshape2)

library(ggplot2)

library(tidyverse)

library(PerformanceAnalytics)

library(psych)

library(dplyr)

library(GGally)
```

6.6.2. Appendix 2: R code for final clofazimine model

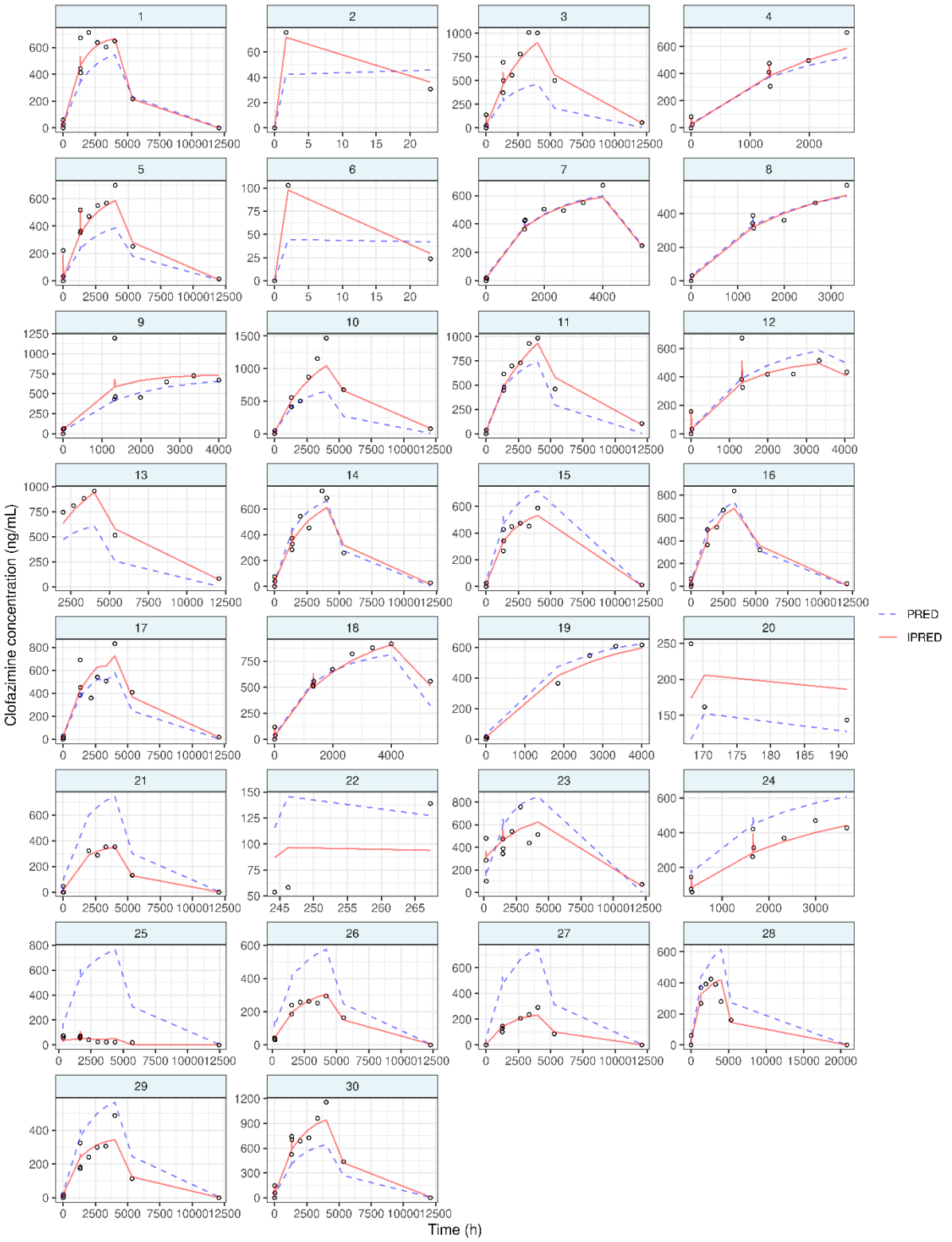
```
ini({
  lka <- fix(-0.4)
  lcl <- 1.92220362629715
  lvc <- 7.46743390838864
  lvp <- 9.1211857006886
  lq <- 3.73105017747415
  ltlag <- fix(-0.478)
  add.err <- c(0, 16.4329636428777)
  prop.err <- c(0, 0.198078971621789)
  covwtPow1 <- fix(0.75)
  covwtPow2 <- fix(1)
  covwtPow3 <- fix(1)
  covwtPow4 <- fix(0.75)
  eta.cl ~ 0.468559376352451
  eta.vc ~ 1.38597556490058
  eta.vp ~ 0.181715057553212
  eta.q ~ 0.514266704922081
})
model({
        ka <- exp(lka)
        cl <- exp(lcl + eta.cl + logWT * covwtPow1)
        vc <- exp(lvc + eta.vc + logWT * covwtPow2)
        vp <- exp(lvp + eta.vp + logWT * covwtPow3)
        q <- exp(lq + eta.q + logWT * covwtPow4)
        alag1 <- exp(ltlag)
        d/dt(depot) = -ka * depot
        d/dt(central) = ka * depot - cl/vc * central - q/vc * central +    q/vp * peri1
        d/dt(peri1) = q/vc * central - q/vp * peri1
        alag(depot) = alag1
        cp = central/vc
        cp ~ prop(prop.err) + add(add.err)
})
```

Run No	Model Description	Reference model	AIC	OFV	Delta AIC	Delta OFV	Comments
1	1CMPT, ETA [CL, V_c], Combined RV	Run 1	3418.72	2879.09	0	0	-
2	2CMPT, ETA [CL, V_c], Combined RV	Run 1	3313.39	2769.75	-105.33	-109.34	Decrease in OFV/AIC, High RSE of K_a
3	3CMPT, ETA [CL, V_c], Combined RV	Run 1	3363.28	2815.65	49.89	45.9	Decrease in OFV/AIC
4	Add IIB on K_a, ETA [CL, V_c, K_a], Combined RV	Run 1	3350.48	2804.85	37.09	35.1	No decrease in OFV/AIC
5	Change to proportional RV	Run 1	4407.65	3866.01	1094.26	1096.26	Failed covariance
6	Change to additive RV	Run 1	3458.62	2916.99	145.23	147.24	No decrease in OFV/AIC
7	Change to log-normal distributed RV	Run 1	3276.33	2734.7	-37.06	-35.05	High RSE of all parameters
8	Introduce tranist compartmemt absorption model	Run 1	3373.92	2832.28	60.53	62.53	No decrease in OFV/AIC
9	Introduced absorption model with lag time 1	Run 1	3325.15	2783.52	11.76	13.77	No decrease in OFV/AIC
10	Introduced absorption model with lag time 2	Run 1	3372.6	2830.97	59.21	61.22	No decrease in OFV/AIC
11	Introduced correlation between CL and V_c	Run 9	3360.77	2817.14	35.62	33.62	Decrease in OFV/AIC
12	Add IIV on V_p, ETA [CL, V_c, V_p]	Run 11	3418.71	2875.08	57.94	57.94	No decrease in OFV/AIC
13	Add IIV on Q, ETA [CL, V_c, Q]	Run 11	3284.65	2741.01	-76.12	-76.13	Decrease in OFV/AIC
14	Add IIV on V_p, ETA [CL, V_c, V_p, Q]	Run 13	3274.03	2728.39	-10.62	-12.62	Decrease in OFV/AIC
15	Introduce full-block omega matrix	Run 14	3304.78	2747.15	30.75	18.76	No decrease in OFV/AIC
16	Introduced correlation between CL and V_c	Run 14	3282.55	2734.92	8.52	6.53	Decrease in OFV/AIC
17	Change to proportional RV	Run 14	3460.62	2916.99	186.59	188.6	No decrease in OFV/AIC
18	Change to additive RV	Run 14	5095.55	4551.92	1821.52	1823.53	No decrease in OFV/AIC
19	Change to log-normal distributed RV	Run 14	4906.78	4363.14	1632.75	1634.75	No decrease in OFV/AIC

6.6.4. Appendix 4: Continuous covariates matrix plot



6.6.5. Appendix 5: Individual patient clofazimine concentration plots



6.6.6. *Appendix 6: Clofazimine half life*

R code for estimating half-life

```
**************************************************************************
# Calculate k20, k23, k32
        mutate(k20 = cl/vc) %>%
        mutate(k23 = q/vc) %>%
        mutate(k32 = q/vp) %>%

# Calculate SUM
        mutate(sum = k20 + k23 +k32 ) %>%

 # Calculate ROOT
        mutate(root = sqrt(sum * sum - 4 * k32 * k20)) %>%

# Calculate alpha and beta
        mutate(alpha = 0.5 * (sum + root)) %>%
        mutate(beta = 0.5 * (sum - root)) %>%

# Calculate t12_alpha and t12_beta
        mutate(t12_alpha = 0.693/alpha) %>%
        mutate(t12_beta = 0.693/beta) %>%
**************************************************************************
```

Table of clofazimine half-life ranges for 50mg,100mg and 200 mg doses

Half-life	minimum	Lower Quartile	Median	Upper Quartile	maximum	dose
Distribution phase (days)	0.01	0.28	0.61	1.31	15.54	50mg
Elimination phase (days)	2.82	27.01	44.48	77.46	708.81	
Distribution phase (days)	0.01	0.29	0.61	1.37	15.70	100mg
Elimination phase (days)	4.93	28.04	45.25	72.98	793.84	
Distribution phase (days)	0.01	0.26	0.61	1.31	48.18	200mg
Elimination phase (days)	2.88	27.57	44.85	76.16	982.42	

6.6.7. Appendix 7: Time above MIC at varying protein binding assumptions

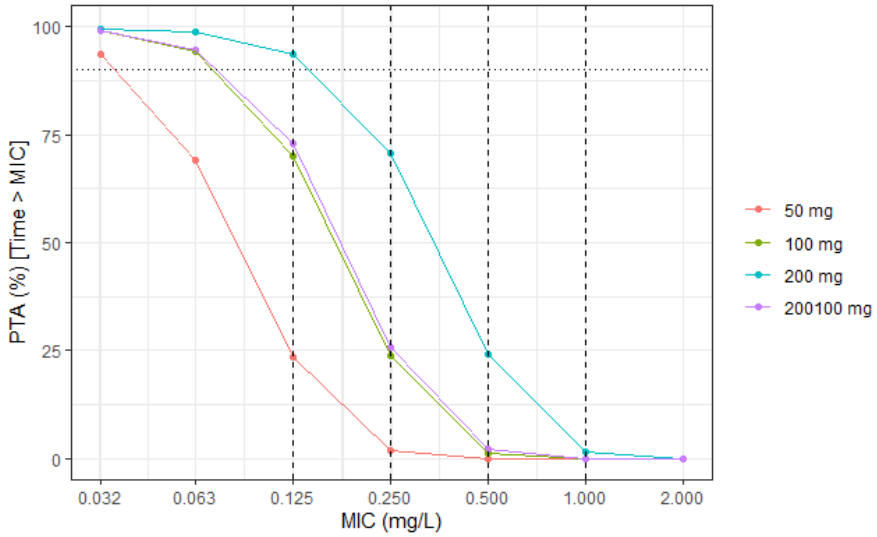


Figure S8.1: % fT above MIC for four dosing approaches at an assumed 85% protein binding .

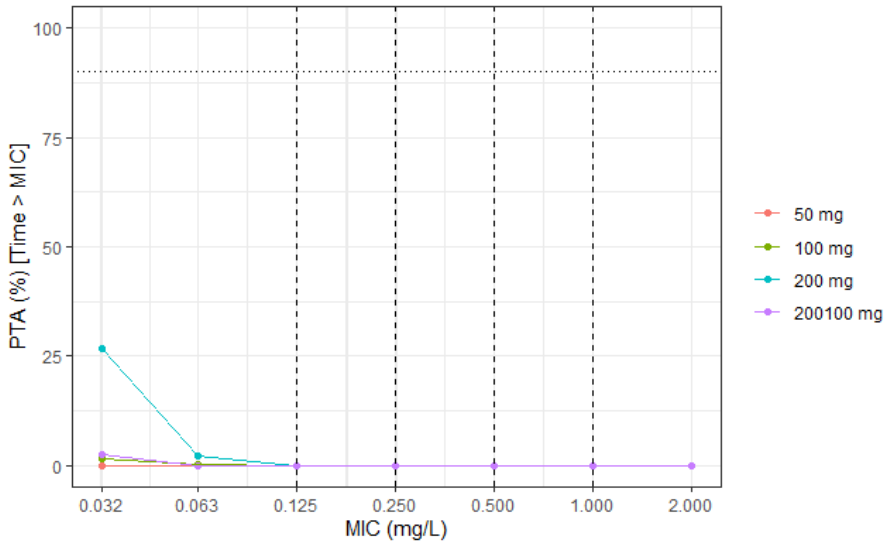


Figure S8.2: % fT above MIC for four dosing approaches at an assumed 99% protein binding.

Chapter 7: Bedaquiline population pharmacokinetics and probability target attainment in adults on BPaL-based treatment for rifampicin-resistant tuberculosis.

7.1. Introduction

This chapter reports on the study methods and results of the population pharmacokinetics of bedaquiline and its probability target attainment. The results section describes the participants of the study, the pharmacokinetic data that was used and the population pharmacokinetic model building. The intermediate model building steps detail out the structural, statistical and covariate model building and evaluation of the final linezolid population pharmacokinetic model. The parameter estimates derived from the final model are presented, including a discussion on how they compare with those from previously published papers. Bedaquiline MICs in the TB-PRACTECAL clinical trial (72) are presented and used in interpreting the probability of achieving the PKPD target.

7.2. Methods

7.2.1. Study design

This was a sub study nested in a randomised controlled trial in patients with rifampicin resistant tuberculosis. Participants received bedaquiline 400mg daily for 2 weeks then 200mg three times a week for 22 weeks as part of each of the three investigational regimens. BPaL arm consisted of the bedaquiline, pretomanid 200mg daily for 24 weeks and tapered dose linezolid 600mg daily for 16 weeks then 300mg for 8 weeks. Clofazimine 100mg daily for 24 weeks was added in BPaLC arm or Moxifloxacin 400mg daily for 24 weeks in BPaLM arm. Blood samples were collected on Day 1 (0, 2 and 23 hours), Weeks 8 (predose, 6.5 and 23 hours), 12, 16, 20, 24, 32 and 72 post randomisation visits. Drug concentrations were quantified in a GCP laboratory using a high-performance liquid chromatography-tandem mass spectrometry. The lower limit of quantification for bedaquiline was 20ng/mL.

7.2.2. Pharmacometric analysis

nlmixr2, an open-source R package was used for population PK modelling and simulation estimation. R v4.4.0 was used for dataset creation, data exploration and generation of tables and plots (see appendix 1 for list of packages used). The PopPK for bedaquiline was analysed using a non-linear mixed effect modelling approach. The first-order conditional estimation with interaction (FOCE-I) algorithm in nlmixr2 was used. Inter-individual variability (IIV) at the parameter level and residual variability (RV) at the observation level made up the mixed effects analysis.

7.2.3. Structural model

The PopPK study first explored basic model structure based on the observed plasma concentration data. One-, two-, three- and four-compartment linear models were evaluated respectively with combined, proportional, additive and log-transformed residual error models. Finally, random effects on clearance (CL), central and peripheral volume of distribution (V) and intercompartmental clearance with correlation were included in the model. Fixed transit compartment models were explored.

7.2.4. Covariate model

A covariate matrix of continuous variables including age, weight, BMI, FFM, BUN, ALT, AST, TP, CLCR and eta estimates on clearance and volume of distribution from the base model explored correlation as well as covariate collinearity. Association between categorical covariates (sex, HIV status, race, treatment) and etas on clearance and volume were also independently explored. Allometric scaling on weight, BMI, FFM were applied to both volume of distribution and clearance. The coefficients of the power model were fixed to 1 for Vc, Vp and 0.75 for CL, Q. The selected covariates underwent stepwise forward inclusion ($P<0.05$, $\Delta OFV > 3.84$) and backward elimination ($p<0.001$, $\Delta OFV > 10.83$) to select those that would improve the model fit significantly.

7.2.5. Model evaluation

Goodness-of-fit plots were used to assess how well the model predicted individual and population values closely matched the observed PK data. Model validation was also performed using visual predictive check (VPC) plots and non-parametric bootstrapping. The shrinkage, relative standard error, and variability value including omega and sigma values were also used to assess the precision and robustness of the model.

7.2.6. Minimum Inhibitory Concentration (MIC)

Early morning sputum samples were collected from each trial participant and cultured in liquid medium in Mycobacterial Growth Indicator Tube (MGIT) system (Becton Dickinson). Minimum inhibitory concentrations were determined from a routine testing concentration set (2, 1, 0.5, 0.25, 0.125 mg/L) in MGIT; testing was performed using a lower (0.063, 0.032 and 0.0016 mg/L) or higher (1, 2, 4 and 8 mg/L) testing concentration if required. The results from all participants from the TB-PRACTECAL trial were summarised by country of enrolment and the median and interquartile range reported. Previously published bedaquiline MIC data was used where PTA targets used a different methodology to MGIT.

7.2.7. Probability of Target Attainment

Comparing exposure-response and constant-drug effect models demonstrated that bedaquiline activity is concentration dependent (118). Furthermore, bedaquiline $AUC_{0\text{-}24}$ /MIC exposure/susceptibility ratios are associated with sputum culture conversion (119). $AUC_{0\text{-}24}$/MIC targets of 175.5, 118.2 and 74.6 are associated with two-month culture conversion, six-month culture conversion and 24-month successful treatment outcomes respectively (120).

Using 2,000 Montecarlo simulations, the probability of attaining the AUC/MIC targets at week 4 and week 8 on treatment were calculated for the MIC range observed in the TB-PRACTECAL trial (0.032 – 2 mg/L). Since bedaquiline is highly protein bound and MICs are not corrected for albumin binding in broth and nonspecific binding to plastics, total drug concentration was used (121).

7.3. Results

7.3.1. Study population

Table 7.1: baseline characteristics of study participants (n = 94)

Characteristic	Total
Female, n (%)	34 (36.2)
Age, years (range)	36 (19-71)
Race, n (%)	
Other	1(1)
Asian	1 (1)
Black	52 (55)
Caucasian	40 (43)
HIV status, n (%)	
positive	39 (42)
negative	54 (57)
Unknown	1(1)
Regimen, n (%)	
BPaLM	38 (40)
BPaLC	30 (32)
BPaL	26 (28)
BMI, Kg/m2	19.7 (14.3 – 47.2)
Fat Free Mass, kg	45.5 (28.6 – 75.5)
BUN (mmol/L)	3.6 (1.7 – 8.5)
ALT (IU/L)	19 (4 – 113)
AST (IU/L)	22 (4 – 82)
ALP* (IU/L)	67 (37 – 132)
Albumin* (g/L)	44 (36 – 49)
Total protein (g/L)	77 (61 – 118)
Creatinine (mcrmol/L)	66 (35 – 111)
Creatinine clearance (mL/min)	105.4 (43.4 – 243.8)

Median (min-max) if not stated otherwise. * n=39

BMI= body mass index, ALT= alanine transaminase, AST= aspartate aminotransferase, ALP=alkaline phosphatase, BUN= blood urea nitrogen

94 study participants (36% female) with a median age of 34 years (range: 19 – 71 years) see table 7.1, contributed 952 timed plasma samples which upon bioanalysis were included in the bedaquiline PopPK dataset. 66 samples were collected before the first dose, and of the 886 post first dose samples, 65 (7% of samples during treatment) were below the limit of quantification.

The observed concentrations of bedaquiline ranged from 25.31 to 7498.8 ng/ml. The median trough concentration was 1028.13 ng/ml, with an interquartile range of 609.58 – 1504.68 ng/ml (see Figure 7.1).

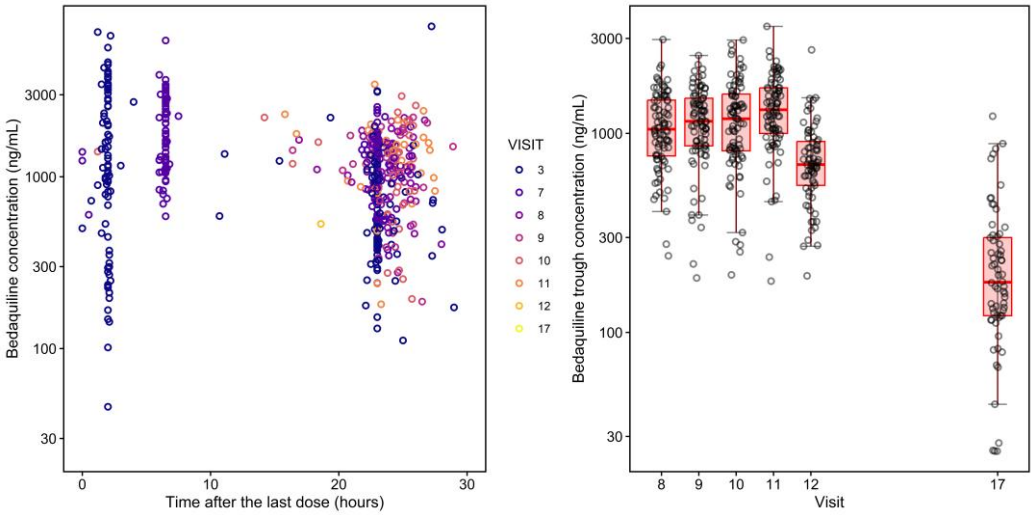


Figure 7.1: Plots of observed bedaquiline concentrations by time after last dose on the left. Trough (visits 8-11 [weeks 12, 16, 20, 24]) and post treatment completion (visits 12 [week 32] and 17 [week 72]) concentrations by time after first dose aggregated by study visit number (right). The pink box represents the interquartile range with the red horizontal line representing the median while the whiskers represent the 5th and 95th percentiles.

The observed pk plots for each individual patient are shown in supplementary appendix 6.

7.3.2. Structural and variability model

A three-compartment model with an OFV of 12294.56 was selected as the best to characterise the bedaquiline observed PK data. The model is represented by figure 7.2 and includes fixed transit compartments from a previous publication (44) as the study sampling scheme was unlikely to adequately capture the complex bedaquiline absorption phase. Random effects on clearance, central volume of distribution,

peripheral volume of distribution and inter-compartmental clearance were included in the model to explain the inter-individual variability. Combined residual error model was used for the unexplained variability as it performed better than additive (OFV = 2900.81), proportional (OFV = 3608.12) and log-transformed additive (OFV = 4582.03) models. The summary of model evaluation results with OFVs and delta ΔOFVs are in supplementary appendix 3 [models 1-20].

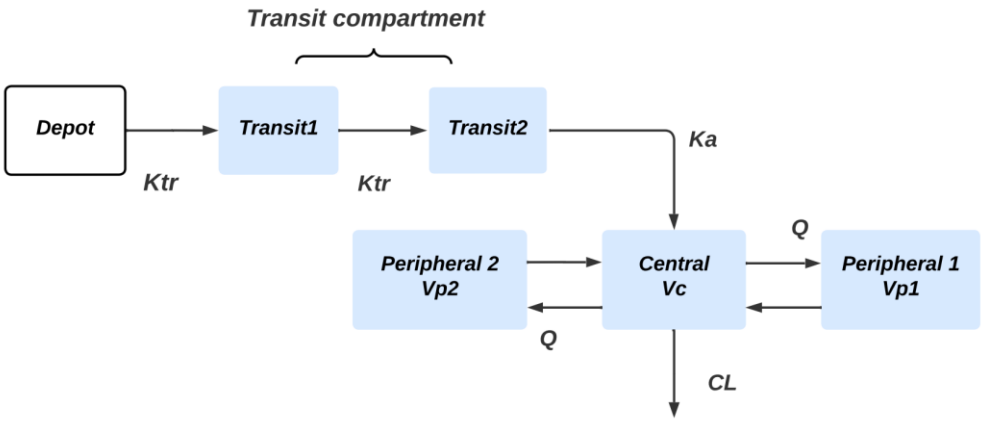


Figure 7.2: Schematic representation of the structural bedaquiline model.

Ktr = transfer rate constant, Ka = absorption rate constant, Vc = central compartment volume, CL = clearance, Q = intercompartmental clearance, Vp = peripheral compartment volume

The chosen base model's r code is in appendix 2 and the ordinary differential equations [7.1] are as follows:

$$\frac{dA_{depot}}{dt} = -K_{tr} \times A_{depot}(t)$$

$$\frac{dA_{transit1}}{dt} = K_{tr} \times A_{depot} - K_{tr} \times A_{transit1}(t)$$

$$\frac{dA_{transit2}}{dt} = K_{tr} \times A_{transit1}(t) - K_a \times A_{transit2}(t)$$

$$\frac{dA_{central}}{dt} = K_a \times A_{transit2} - \frac{CL}{V_c} \times A_{central}(t) - Q/V_c * A_{central} + Q/V_p * A_{peripheral1}(t) - Q_2/V_c * A_{central} + Q_2/V_{p2} * A_{peripheral2}(t)$$

$$\frac{dA_{peripheral1}}{dt} = Q/V_c * A_{central} - Q/V_p * A_{peripheral1}(t)$$

$$\frac{dA_{peripheral2}}{dt} = Q_2/V_c * A_{central} - Q_2/V_{p2} * A_{peripheral2}(t)$$

Where A_{depot} is the amount of bedaquiline in the depot compartment, $A_{transit1}$ is the amount in the first transit compartment, $A_{transit2}$ is the amount in the second transit compartment, $A_{central}$ is the amount of bedaquiline in the central compartment, K_{tr} is the absorption rate constant for the transfer of bedaquiline from depot to first transit compartment , K_a is the absorption rate constant for the transfer of bedaquiline from second transit compartment to central compartment, $A_{peripheral1}$ and $A_{peripheral2}$ are the amounts of bedaquiline in the first and second peripheral compartments. CL/F is the apparent clearance of bedaquiline, V_c/F and V_p is the apparent volume of distribution of bedaquiline for central and peripheral compartment. Q is the inter-compartmental clearance.

7.3.3. Covariate model

BMI was the only covariate with significant impact at p<0.001 in the backward step during covariate selection, so the final model included BMI allometric scaling (OFV 12282). However, inclusion of black race on clearance and volume, age and BPaL arm on clearance resulted in significant model improvement at p<0.05 during the forward steps. A summary of covariate model evaluation results with OFVs and delta ΔOFVs is in

supplementary appendix 4 [Cov Runs 1-51] and a matrix plot of chosen model's covariates corelations in appendix 5.

7.3.4. Final model evaluation

Goodness-of-fit plots for the final PopPK model showed no significant bias from the unity line in both PRED VS DV and IPRED VS DV, indicating that the model predicted individual and population values closely matched the observed PK data (Figure 7.3).

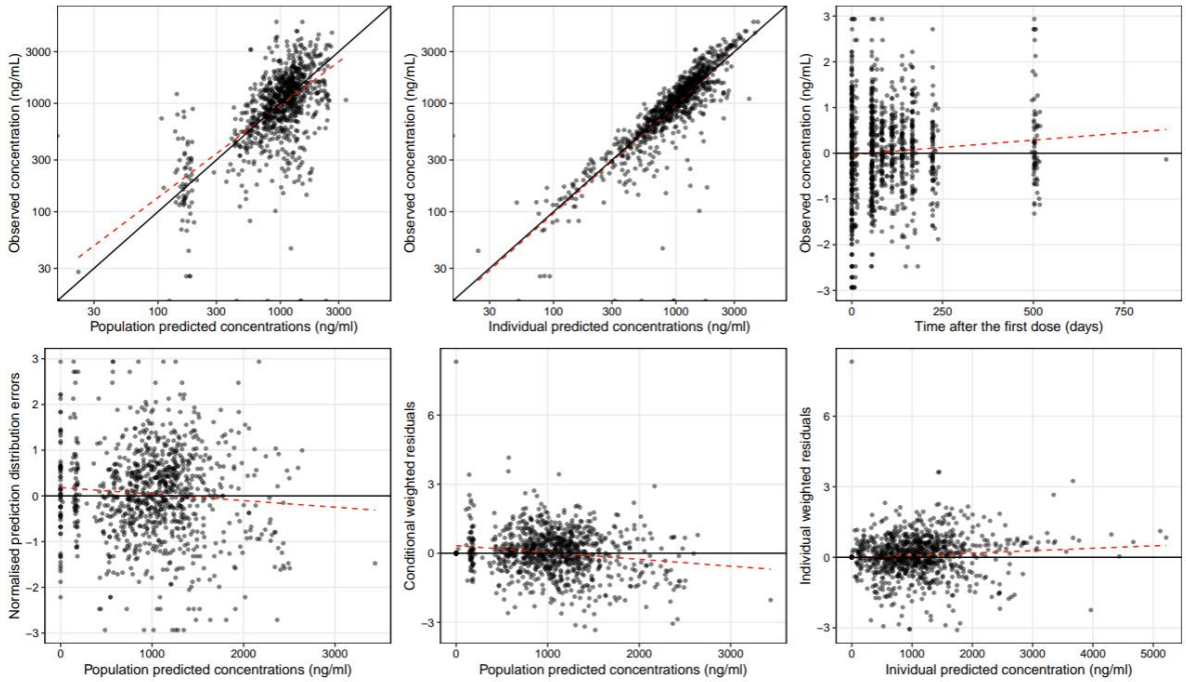


Figure 7.3: Final bedaquiline model goodness of fit plots clockwise from top left: DV vs PRED, DV vs IPRED, DV vs TAFD, IWRES vs IPRED, CWRES vs PRED, NPDE vs PRED

Model validation using visual predictive check (VPC) plots visually showed the predictive accuracy of the final model (figure 7.4).

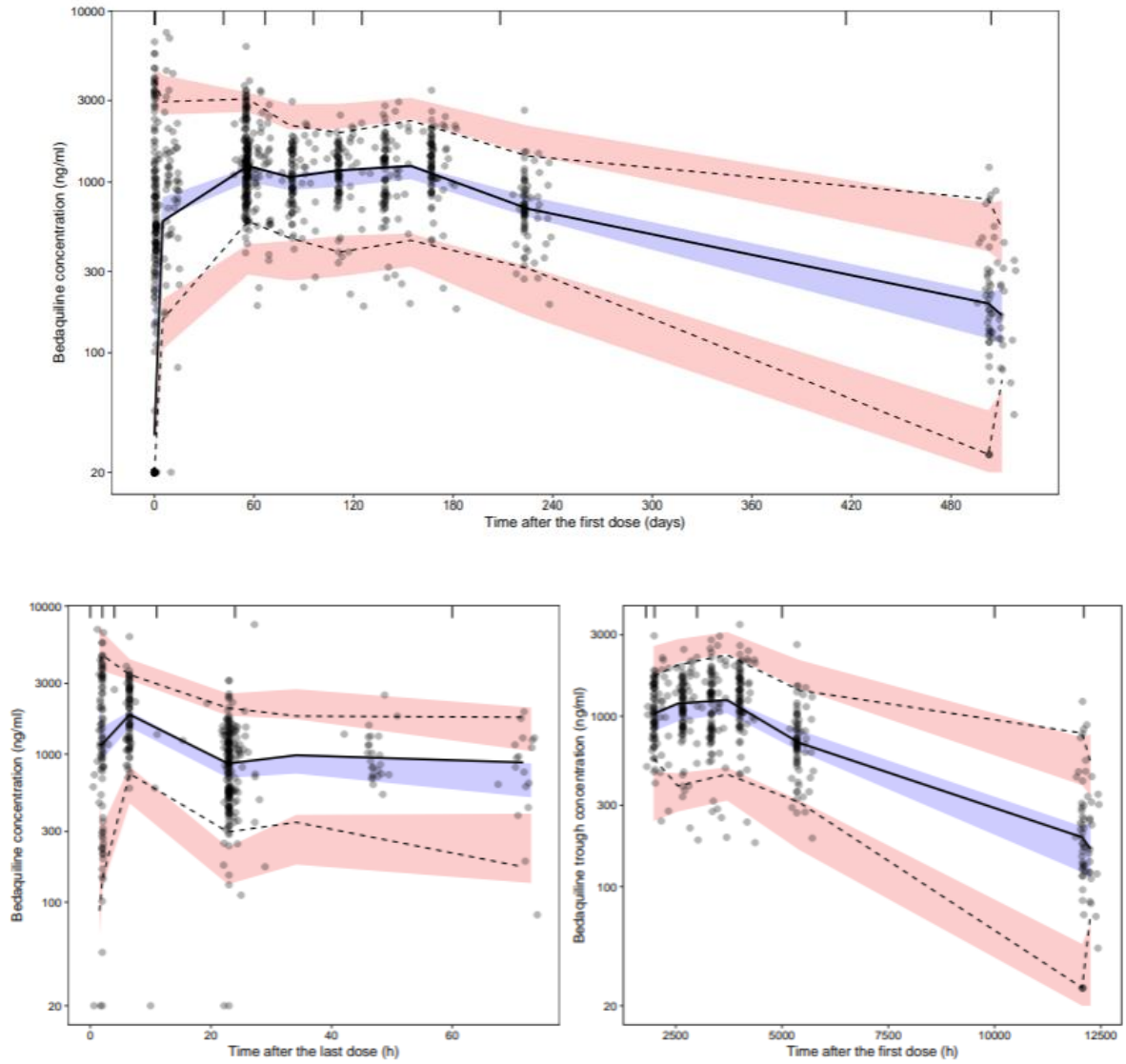


Figure 7.4: Bedaquiline final model VPC plots. The black circles in the figure represents the observed plasma concentrations. Solid black line and dash black line represent the median and 95% confidence interval of observations, respectively. The purple area represents a simulation-based 95% confidence interval for the median. Simulated prediction intervals for 5th and 95th percentiles are presented with pink.

The final model parameters are shown in table 7.2 below.

Table 7.2: The final bedaquiline model parameters

Parameters	Estimate (%RSE [a])	95% CI [b]	Bootstrap Median [95% CI [c]]
MAT [d], fraction of 6 hours	0.66 (Fixed)	-	-
FR	0.47 (Fixed)	-	-
$CL/F(L/h)$	1.93 (7.03)	1.76 - 2.11	1.93 [1.7 - 2.15]
$V_c/F(L)$	103 (2.78)	79.7 - 132	102 [71.7 - 131]
V_p/F (L)	6510 (0.57)	5900 - 7180	6520 [5850 - 7490]
$Q(L/h)$	7.49 (5.98)	5.92 - 9.49	7.54 [5.74 - 9.46]
V_{p2} (L)	98.9 (7.85)	48.8 - 201	93.7 [42 - 177]
Q_2 (L/h)	7.02 (9.98)	4.8 - 10.3	6.92 [4.44 - 10.5]
BMI effect on CL, Q, Q_2 [e]	0.75 (fixed)	-	-
BMI effect on V_c, V_p, V_{p2}	1 (fixed)	-	-
Between subject variability [f]	Estimate	Shrinkage (%)	Bootstrap Median [95% CI]
IIVCL(%)	43.1	8.06	43.5 [31.8 - 57]
IIVV_c(%)	132	10.3	133 [87.4 - 180]
IIVV_p(%)	37.1	16.7	37.2 [27.3 - 49.8]
IIVQ(%)	66.1	15.7	64.7 [44.6 - 92]
Correlation CL-V_c	0.287	-	0.321 [0.243 - 0.413]
Correlation CL-V_p	0.519	-	0.513 [0.362 - 0.585]
Correlation CL-Q	0.374	-	0.403 [0.251 - 0.477]
Correlation V_c-V_p	0.287	-	0.321 [0.243 - 0.413]
Correlation V_c-Q	0.277	-	0.315 [0.23 - 0.456]
Correlation V_p-Q	0.351	-	0.407 [0.149 - 0.49]
Residual Error (σ):			
Proportional	0.321	-	0.322 [0.284 - 0.379]
Additive (mg/L)	0.0595	-	0.0591 [0 - 0.1]

MAT=mean absorption time; FR= fraction of MAT for delay part; CL=clearance; Vc=central volume of distribution; Vp=volume of distribution of peripheral compartment; Q=inter-compartmental clearance

[a] RSE = relative standard error, calculated as 100 × (standard error (SE)/typical value).

[b] 95 % CI = 95 % percentile confidence interval, calculated as point estimate +/- 2 × SE

[c] In the bootstrap, 95 % CI was computed using 2.5th, and 97.5th percentiles of the parameter estimates from a bootstrap with 500 samples.

[d] The final model incorporates the transit absorption model as described in published by Svensson *et al.*, with the population estimates fixed. MAT refers to fraction of time for both delay and 90% complete absorption.

[e] Allometric scaling based on BMI with the power of 0.75 for clearance and inter-compartmental clearance, 1 for volume of distribution is introduced into the final model

[f] BSV % reported in CV scale was calculated as $\sqrt{(e^{\omega 2} - 1)}$ where ω is standard deviation for variability

Correlation coefficient between parameters was calculated as $\frac{\omega_{21}}{\sqrt{\omega_{11}^2}\sqrt{\omega_{22}^2}}$

The estimated primary parameters are on the lower range of the published models, clearance in the identified studies ranged from 1.50L/hr to 4.52 L/hr and the combined volume of distribution from 226 L to 11,438 L as detailed in table 3 below.

Table 7.3: previously published bedaquiline population pharmacokinetic models and estimated primary parameters

Publication	population	clearance (l/hr)	volume (l)	volume P1 (l)	volume P2 (l)	volume P3 (l)
Svensson EM *et al*. 2013 (122)	healthy adults	2.96	17.3	2870	136	N/A
McLeay SC *et al*. 2014 (43)	healthy + MDR-TB	2.78	164	178	3010	7350
Svensson EM *et al*. 2016 (122)	MDRTB	2.62	198	8550	2690	N/A
Zhu H *et al*. 2021 (123)	MDRTB	1.50	1250	4960	N/A	N/A
Zou J *et al*. 2022 (124)	MDRTB	4.52	226	N/A	N/A	N/A
Shao G *et al*. 2023 (120)	MDRTB	3.57	336.97	2839.13	1391.89	N/A

7.3.5. MIC

The distribution of MICs of bedaquiline in baseline pure isolates of M. tuberculosis from 464 TB-PRACTECAL study participants disaggregated by country of enrolment are presented in Figure 7.5. The median MIC was 0.25mg/L and the interquartile range from 0.25 to 0.25mg/L. 99% of the baseline isolates were below the ECOFF (125) and WHO (115) defined critical concentration of 1mg/L. Thirteen of 464 (3%) baseline isolates had a baseline MIC of 1.0mg/L.

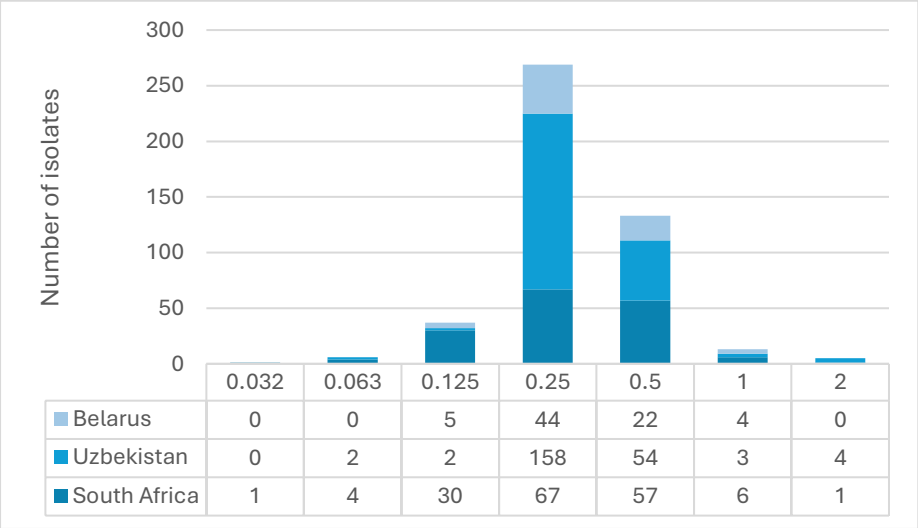


Figure 7.5: Distribution of M.tb baseline isolates across various bedaquiline MICs in the TB-PRACTECAL trial

7.3.6. Target attainment Analysis

At the WHO defined critical concentration(125) on Middlebrook 7H11 of 0.25mg/l, the probabilities of reaching 175.5, 118.2 and 74.6 AUC/MIC targets were 9%, 44% and 87% respectively. As shown in figure 7.6, for the 175.5 target none of the MICs above 0.063mg/L reached the 90% PTA threshold. For the 118.2 target, only up to MIC of 0.125mg/L was the PTA above 90% reached. Despite the long half-life, the bedaquiline PTAs at 4 and 8 weeks are similar.

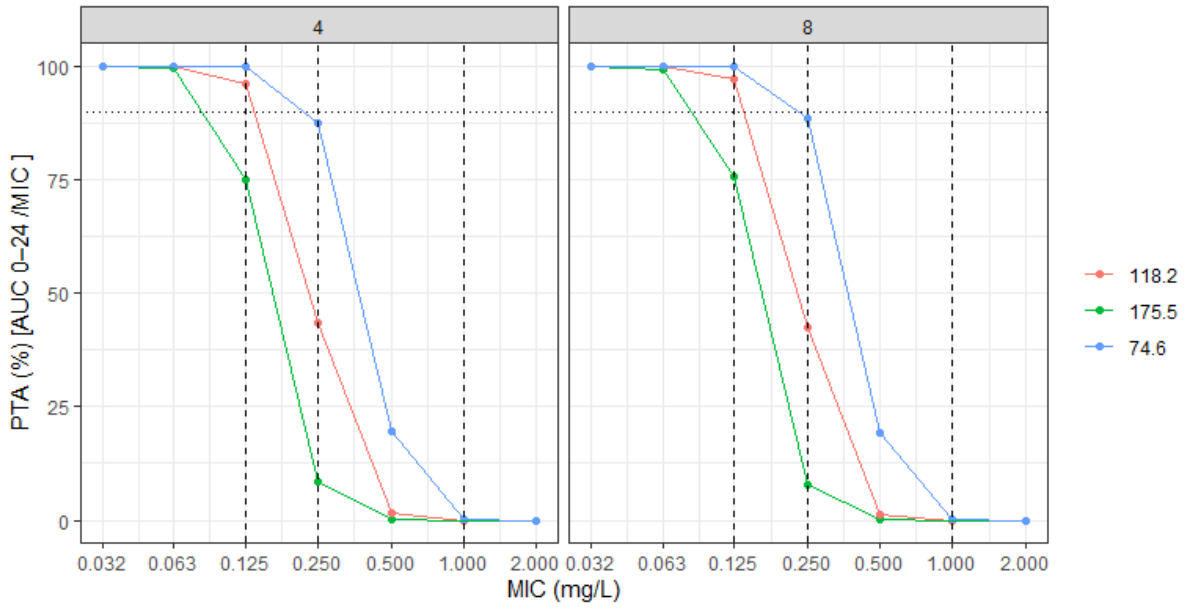


Figure 7.6: Bedaquiline probability AUC/MIC target attainment at week 4 (left) and week 8 (right) comparing the three PKPD targets at the labelled dose of 400mg od for 2 weeks then 200mg thrice a week.

The PTA for the two bedaquiline doses recommended by WHO for the treatment of rifampicin resistant TB were almost identical at week 12 of treatment (figure 7.7)

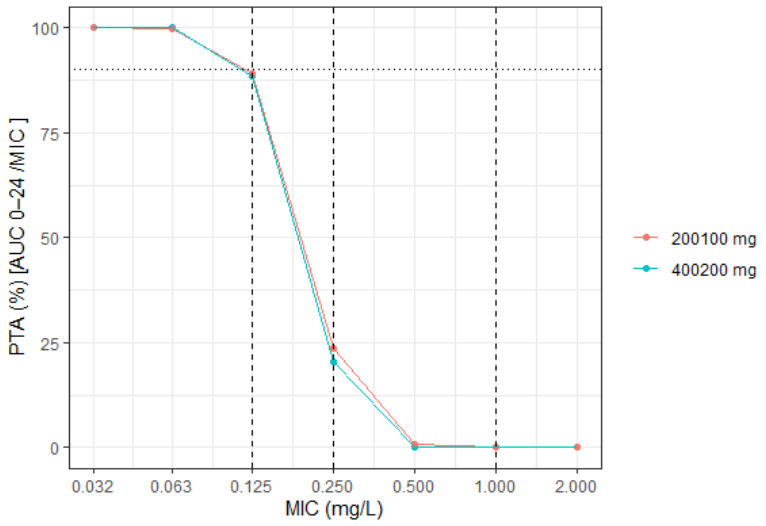


Figure 7.7: Bedaquiline probability AUC/MIC target attainment comparing the 400mg daily for 2 weeks then 200mg thrice a week and ZeNix dose of 200 mg daily for 8 weeks, followed by 100 mg daily doses at week 12.

7.4. Discussion

Bedaquiline population pharmacokinetics in patients with rifampicin resistant tuberculosis from Belarus and South Africa was best described by a three-compartment model with fixed transit compartments. BMI was the only covariate included in the final model. When dosed at the standard 400mg daily for two weeks followed by 200mg three times a week, probability of target attainment above 90% is only achieved for MGIT MICs below 0.063mg/L.

Bedaquiline has a long terminal half-life and was still measurable in most patients in our study more than 48 weeks after treatment completion (figure 7.1). Bedaquiline resistance development is a growing global concern (126). However, resistance has only been reported to have developed during treatment (127) and often associated with cavitary disease and accompanying medications (128). In the TB-PRACTECAL trial, ten recurrences occurred in the BPaL-based regimens (of 400 total participants) and bedaquiline resistance was observed in three of the four participants in the BPaL regimen, and none in the BPaLM and BPaLC regimens (72). However, off-target bedaquiline resistance associated variants in the *mmpR5* (*Rv0678*) gene also results in clofazimine increased MIC (129). Therefore, the role of the post-treatment completion effective bedaquiline monotherapy in resistance development needs further exploration.

At an estimated 1.93 L/hr, the clearance in our study is within the range previously reported in MDR-TB patients (43, 120, 121, 123). Previous bedaquiline pharmacokinetic studies have reported body weight, albumin, race, sex and age as significant covariates (120). In our study, although black race had a significant impact on both clearance and volume at $p<0.05$, only BMI reached the $p<0.001$ threshold (Supplementary Appendix 5). Although total protein was explored as a covariate, albumin was not included in the model as it was not on the panel in one of the study sites resulting in more than 50% of the patients missing this value. Our study population was relatively young, potentially explaining why age wasn't a significant covariate. Of note, as all HIV patients in TB-PRATECAL were put on antiretroviral regimens that consisted of an integrase inhibitor (dolutegravir or raltegravir) and nucleoside reverse transcriptase inhibitors, the finding that HIV status was not a significant covariate, could suggest limited drug-drug interactions. Drug exposure did not vary by treatment regimen, which could also imply limited DDI with clofazimine and moxifloxacin.

The large volume of distribution found in our study (102l, 6520l and 93.7l for central, first and second peripheral compartments respectively), which is within the range of previously reported studies (43, 121, 122), can be explained by the highly lipophilic (cationic amphiphilic) nature of the bedaquiline molecule resulting in extensive tissue distribution (130).

In the absence of established MGIT MIC targets, Middlebrook 7H11 targets were used instead. Over 96% of wildtype M. tuberculosis and non-bedaquiline exposed isolates have a bedaquiline MIC below 0.125mg/L (131), meaning the PTA would have been above 80% for all the targets. However, the mode MIC increases by at least two dilution steps after exposure beyond 90 days (132), raising the question on whether the current dosing is adequate, as at 0.25mg/L the PTA is below 90% for even the lowest target. New studies to establish MGIT MIC targets are needed.

Bedaquiline does not reach steady state throughout the six-month treatment period (133), however the target attainment between week four and week eight did not differ significantly (figure 7.6), confirming previous assertions that drug exposure is relatively stable between weeks four and 24 when the labelled dosing is used (134). Simulating the

200mg daily for 8 weeks followed by 100mg daily as used in the ZeNix trial (135) did not alter the target achievement significantly (figure 7.7).

The study has some limitations mainly related to the fact that it was a sub-study so the sample size was opportunistic and although the optimal design analyses indicated that the timing of the samples were adequate, the sampling was originally optimised for linezolid pharmacokinetics (79). Inherent to limitations of PTAs of individual drugs while clinically used as part of a regimen, the actual targets may be modified by the accompanying drugs, but this could not be adjusted for.

7.5. Conclusion

Bedaquiline exposure at the labelled dose is adequate for most South African and Belarussian patients on a BPaL based regimens.

7.6. Chapter 7 supplementary Appendices

7.6.1. Appendix 1: Used r packages

```
library(rxode2)

library(nlmixr2)

library(reshape2)

library(ggplot2)

library(tidyverse)

library(PerformanceAnalytics)

library(psych)

library(dplyr)

library(GGally)
```

7.6.2. Appendix 2: R code for final bedaquiline model

```
ini({
  lcl <- 0.681097255280442
  lvc <- 4.65688200230175
  lvp <- 8.81768483890911
  lq <- 2.0425014630809
  lvp2 <- 4.64344365972148
  lq2 <- 1.99661976997726
  prop.err <- c(0, 0.319868740114323)
  add.err <- c(0, 59.9108193173549)
  eta.cl + eta.vc + eta.vp + eta.q ~ c(0.168705694496233, 0.104703843997953,
    0.92093839782451, 0.0955705277761172, 0.107717960726834,
    0.176302513642733, 0.0815226270983771, 0.180167402280474,
    0.0728389243161003, 0.336841321600005)
})
model({
  cl <- exp(lcl + eta.cl)
  vc <- exp(lvc + eta.vc)
  vp <- exp(lvp + eta.vp)
  q <- exp(lq + eta.q)
  vp2 <- exp(lvp2)
  q2 <- exp(lq2)
  TVMAT <- 0.66
  TVFR <- 0.47
  PHI = log(TVMAT/(1 - TVMAT))
  MAT = 6 * exp(PHI)/(exp(PHI) + 1)
  FR = TVFR
  MTT = MAT * FR
  KAHL = MAT * (1 - FR)/3.3
  ka = log(2)/KAHL
  ktr = 2/MTT
  d/dt(depot) = -ktr * depot
  d/dt(transit1) = ktr * depot - ktr * transit1
  d/dt(transit2) = ktr * (transit1) - ka * transit2
  d/dt(centr) = ka * (transit2) - cl/vc * centr - q/vc * centr +
    q/vp * peri1 - q2/vc * centr + q2/vp2 * peri2
  d/dt(peri1) = q/vc * centr - q/vp * peri1
  d/dt(peri2) = q2/vc * centr - q2/vp2 * peri2
  cp = centr/vc
  cp ~ prop(prop.err) + add(add.err)
})
```

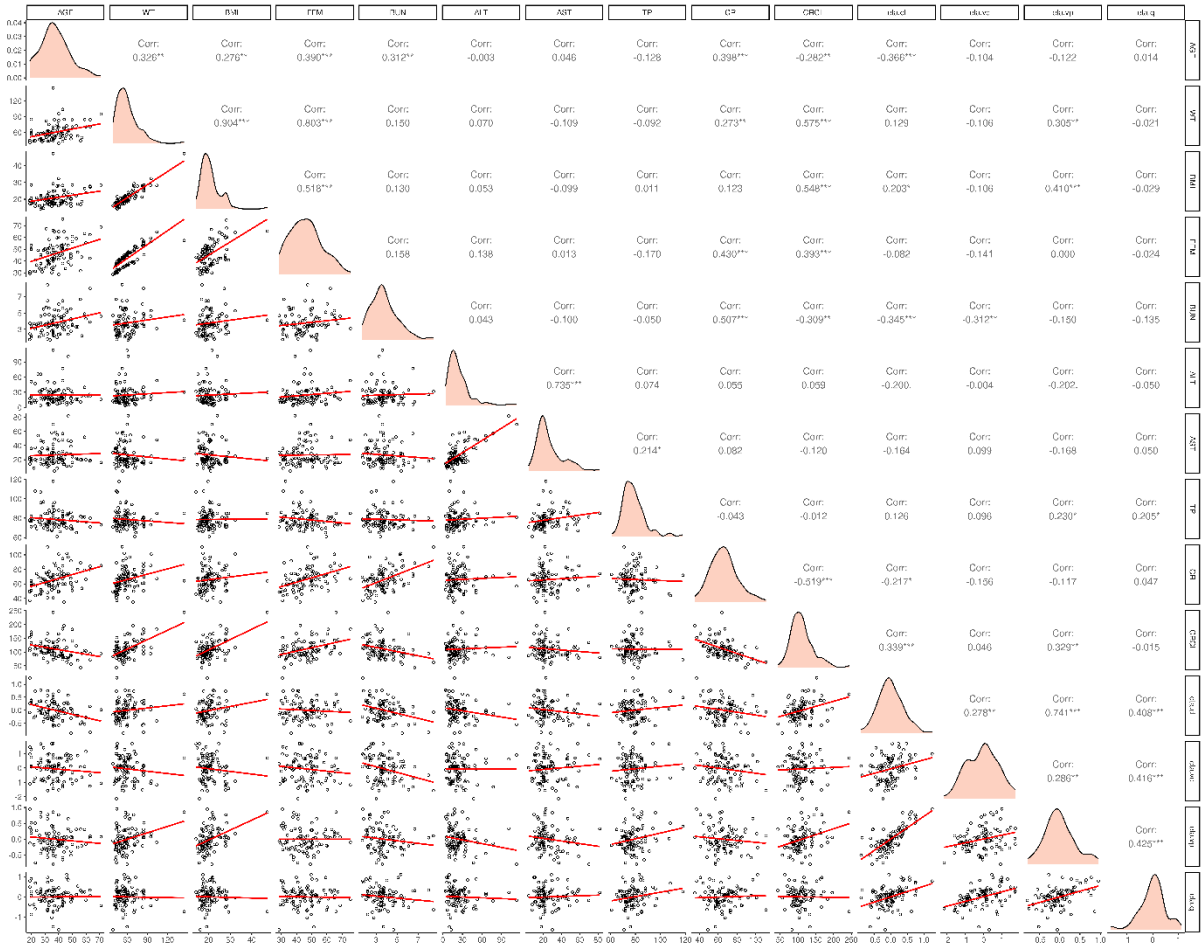
Run No	Model Description	Reference model	AIC	OFV	ΔAIC	ΔOFV	Comments
1	1CMPT, ETA [CL, V_c], Combined RV	Run 1	15048.82	13285.16	0	0	-
2	2CMPT, ETA [CL, V_c], Combined RV	Run 1	14368.21	12600.55	-680.61	-684.61	Decrease in OFV/AIC
3	3CMPT, ETA [CL, V_c], Combined RV	Run 2	14282.23	12510.57	-85.98	-89.98	Decrease in OFV/AIC
4	4CMPT, ETA [CL, V_c], Combined RV	Run 3	14260.82	12485.16	-21.41	-25.41	High RSE on most of parameters
5	Introduce transit compartment	Run 3	14274.91	12505.25	-7.32	-5.32	Decrease in OFV/AIC
6	Change to proportional RV	Run 5	262984.5	261216.8	248709.56	248711.57	Failed covariance
7	Change to additive RV	Run 5	14842.64	13074.98	567.73	569.73	No decrease in OFV/AIC
8	Change to log-normal distribution RV	Run 5	15286.57	13649.4	1011.66	1144.15	No decrease in OFV/AIC
9	Add IIV on V_p, ETA [CL, V_c, V_p]	Run 5	14227.52	12455.86	-47.39	-49.39	Decrease in OFV/AIC
10	Add IIV on Q, ETA [CL, V_c, Q]	Run 5	14163.89	12392.23	-111.02	-113.02	Decrease in OFV/AIC
11	Add IIV on V_p2, ETA [CL, V_c, V_p2]	Run 5	14310.8	12539.14	35.89	33.89	No decrease in OFV/AIC
12	Add IIV on $Q2$, ETA [$CL, V_c, Q2$]	Run 5	14263.27	12491.61	-11.64	-13.64	Decrease in OFV/AIC
13	Add IIV on Q, ETA [CL, V_c, V_p, Q]	Run 10	14093.44	12319.78	-70.45	-72.45	Decrease in OFV/AIC
14	Add IIV on V_p2, [CL, V_c, Q, V_p2]	Run 10	14146.54	12372.88	-17.35	-19.35	Decrease in OFV/AIC
15	Add IIV on $Q2$, [$CL, V_c, Q, Q2$]	Run 10	14159.75	12386.09	-4.14	-6.14	Decrease in OFV/AIC
16	Add IIV on V_p2, [CL, V_c, V_p, Q, V_p2]	Run 13	14265.58	12489.92	172.14	170.14	No decrease in OFV/AIC
17	Add IIV on $Q2$, [$CL, V_c, V_p, Q, Q2$]	Run 13	14095.81	12320.15	2.37	0.37	No decrease in OFV/AIC
18	Introduce full-block omega	Run 13	14080.21	12294.56	-13.23	-25.22	Decrease in OFV/AIC
19	Change to proportional RV	Run 18	5488628	5486844	5474547.6	5474549.59	Failed covariance
20	Change to additive RV	Run 18	14656.11	12872.45	575.9	577.89	No decrease in OFV/AIC

7.6.4. Appendix 4: Covariate model selection – objective function values

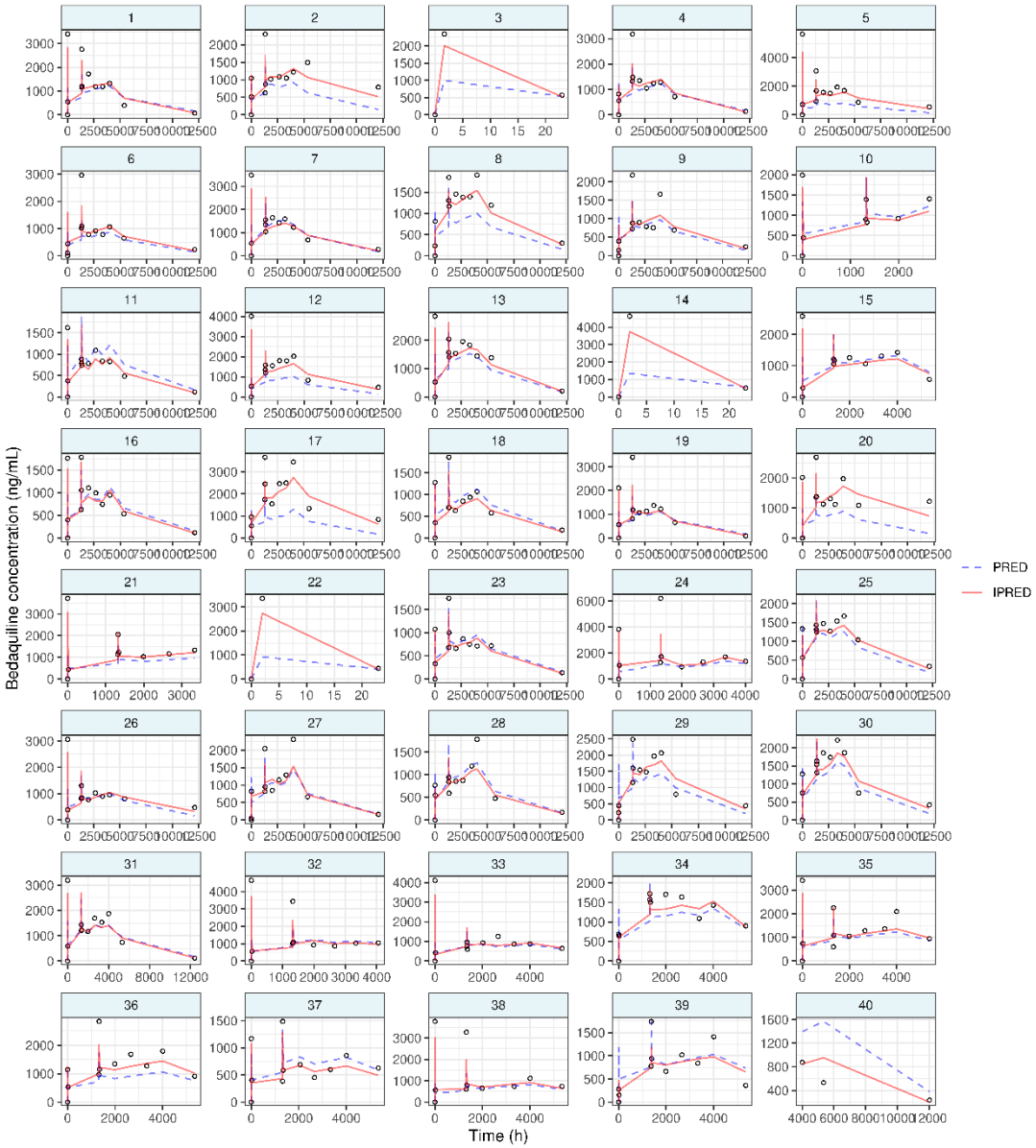
Run No	Model Description	Reference model	OFV	dOFV	Significant (Y/N)
1	Base model	Run 1	12294.56	-	-
	Cycle 1				
2	Introduce allometric scalling (WT)	Run 1	12299.98	-6.14	N
3	Introduce allometric scalling (BMI) [Final Model]	Run 1	12282	-12.56	Y
4	Introduce allometric scalling (FFM)	Run 1	12325.12	-5.02	N
	Cycle 2				
5	Introduce AGE on CL	Run 3	12275.54	-6.46	Y
6	Introduce BUN on CL	Run 3	12277.1	-4.9	Y
7	Introduce ALT on CL	Run 3	12277.4	-4.6	Y
8	Introduce AST on CL	Run 3	12280.22	-1.78	N
9	Introduce AST on CRCL	Run 3	12285.38	3.38	N
10	Introduce TP on CL	Run 3	12281.87	-0.13	N
11	Introduce FEMALE on CL	Run 3	12278.91	-3.09	N
12	Introduce CAUCASIAN on CL	Run 3	12274.69	-7.31	Y
13	Introduce BLACK on CL	Run 3	12274.28	-7.72	Y
14	Introduce HIV on CL	Run 3	12281.45	-0.55	N
15	Introduce TREATMENT1 on CL	Run 3	12281.42	-0.58	N
16	Introduce TREATMENT2 on CL	Run 3	12276.5	-5.5	Y
17	Introduce TREATMENT3 on CL	Run 3	12274.85	-7.15	Y
18	Introduce AGE on Vc	Run 3	12281.73	-0.27	N
19	Introduce TP on Vc	Run 3	12281.87	-0.13	N
20	Introduce FEMALE on Vc	Run 3	12286.79	4.79	N
21	Introduce CAUCASIAN on Vc	Run 3	12275.92	-6.08	Y
22	Introduce BLACK on Vc	Run 3	12274.42	-7.58	Y
23	Introduce HIV on Vc	Run 3	12288.69	6.69	N
24	Introduce TREATMENT1 on Vc	Run 3	12282.78	0.78	N
25	Introduce TREATMENT2 on Vc	Run 3	12283.6	1.6	N
26	Introduce TREATMENT3 on Vc	Run 3	12285.11	3.11	N
	Cycle 3				
27	Introduce AGE on CL	Run 22	12268.28	-6.14	Y
28	Introduce BUN on CL	Run 22	12269.55	-4.87	Y
29	Introduce ALT on CL	Run 22	12269.76	-4.66	Y
30	Introduce CAUCASIAN on CL	Run 22	12266.04	-8.38	Y
31	Introduce BLACK on CL	Run 22	12264.8	-9.62	Y
32	Introduce TREATMENT2 on Vc	Run 22	12269.05	-5.37	Y
33	Introduce TREATMENT3 on Vc	Run 22	12267.75	-6.67	Y
34	Introduce CAUCASIAN on Vc	Run 22	12275.8	1.38	N
	Cycle 4				
35	Introduce AGE on CL	Run 31	12258.29	-6.51	Y

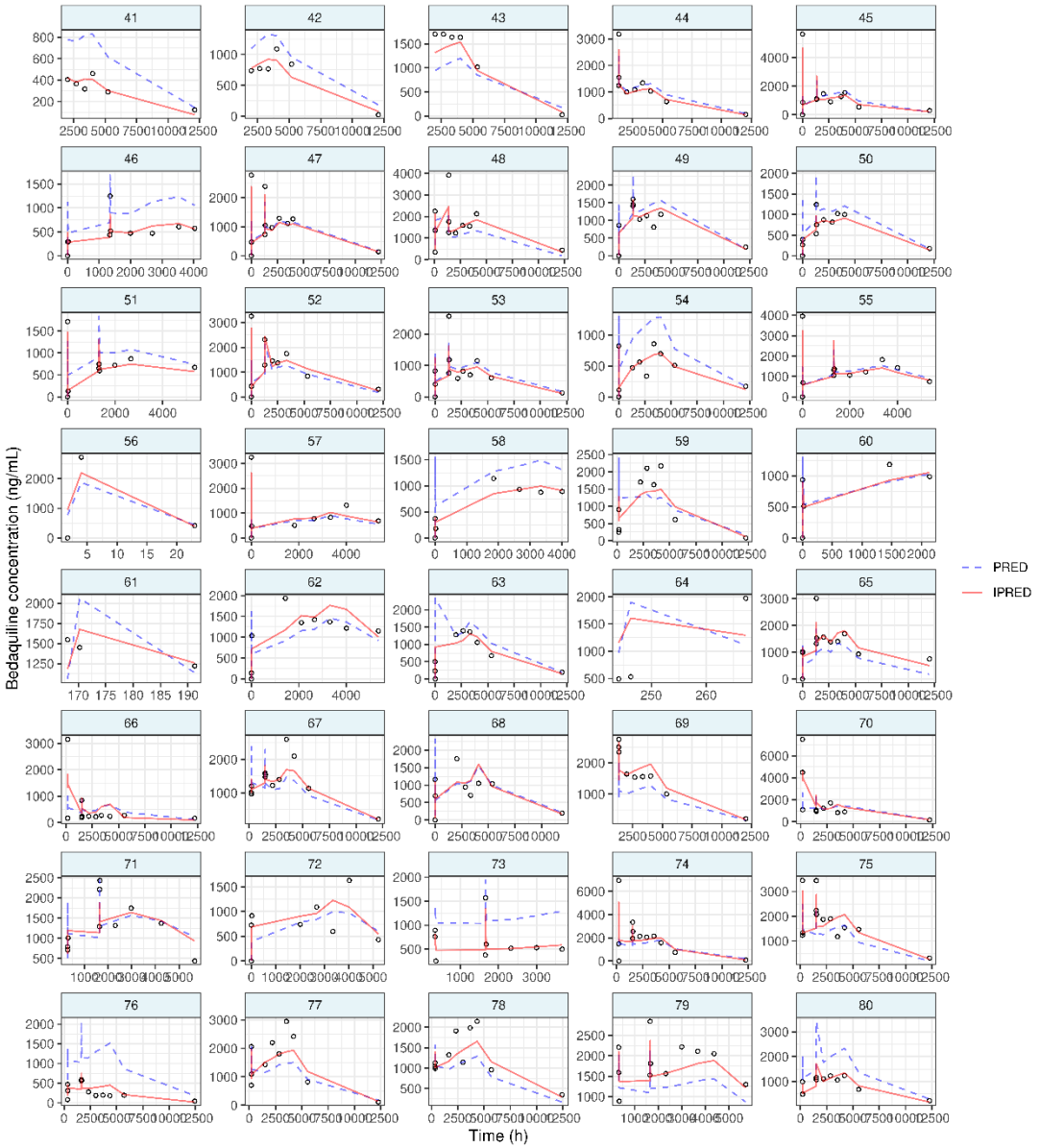
36	Introduce BUN on CL	Run 31	12260.73	-4.07	Y
37	Introduce ALT on CL	Run 31	12260.2	-4.6	Y
38	Introduce CAUCASIAN on CL	Run 31	12264.52	-0.28	N
39	Introduce TREATMENT2 on Vc	Run 31	12260.67	-4.13	Y
40	Introduce TREATMENT3 on Vc	Run 31	12257.66	-7.14	Y
41	Introduce CAUCASIAN on Vc	Run 31	12266.81	2.01	N
	Cycle 5				
42	Introduce AGE on CL	Run 40	12252.36	-5.3	Y
43	Introduce BUN on CL	Run 40	12253.92	-3.74	N
44	Introduce ALT on CL	Run 40	12253.83	-3.83	N
45	Introduce TREATMENT3 on Vc	Run 40	12257.01	-0.65	N
	Stepwise backforward elimination				
46	Remove BMI	Run 42	12268.21	15.85	Y
47	Remove BLACK from Vc	Run 42	12262.44	10.08	N
48	Remove BLACK from CL	Run 42	12262.13	9.77	N
49	Remove AGE from CL	Run 42	12257.66	5.3	N
50	Remove TREATMENT3 from Vc	Run 42	12258.29	5.93	N

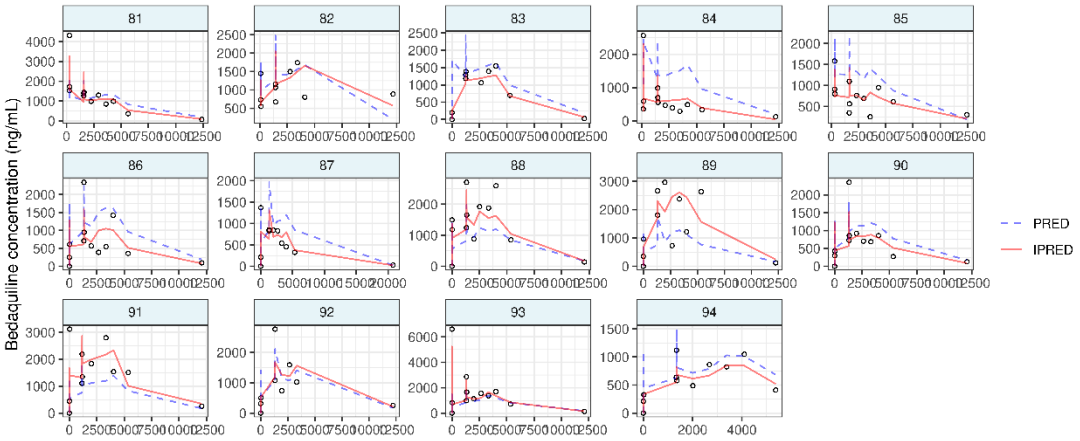
7.6.5. Appendix 5: Continuous variates matrix plot – against etas



7.6.6. Appendix 6: Individual patient bedaquiline concentration plots




Bedaquiline concentration (ng/mL)
Time (h)
PRED
IPRED


81
82
83
84
85
86
87
88
89
90
91
92
93
94
Bedaquiline concentration (ng/mL)
PRED
IPRED

Time (h)

Chapter 8: Overall discussion and conclusions

This chapter summarises the results of the studies conducted, place them in context of the current research on the subject and appraises the studies and findings critically. A reflection on the policy implications of the results as well as future research orientations are presented.

8.1. Summary of research findings

The aim of this thesis was to identify short, effective and safe all oral regimen(s) for the treatment of pulmonary rifampicin resistant tuberculosis by trialling regimens that contained bedaquiline and pretomanid. Below is a summary of the key findings as they relate to the two objectives of this thesis.

Objective 1: *Develop and implement a pragmatic clinical trial for a short, effective and less toxic regimen(s) for rifampicin resistant tuberculosis (TB-PRACTECAL).*

In the first stage of the trial, equivalent to a phase 2, the percentages of patients with culture conversion in liquid medium at 8 weeks after randomization were 77%, 67%, and 46% in the BPaLM, BPaLC, and BPaL groups, respectively and 8%, 6%, and 10% of the patients, respectively, discontinued treatment or died by week 8. All three investigational regimens (BPaLM, BPaLC and BPaL) had therefore passed the pre-defined efficacy and safety thresholds and hence were eligible to progress to stage 2. BPaLM was selected to progress to stage two of the trial (136).

In stage two of the trial, 88% of participants taking the BPaLM regimen had a successful outcome at 72 weeks post randomisation compared to 59% in the standard of care. The unadjusted risk difference was -29.2 % demonstrating both noninferiority and superiority of $p < 0.0001$. The unadjusted risk ratio was 0.29. The BPaLC and BPaL regimens groups had 77% and 86% successful outcomes respectively. The proportion of patients with at least one grade 3 or above adverse event or serious adverse event were 48%, 23%, 30% and 24% in standard of care, BPaLM, BPaLC and BPaL groups respectively. All studied short all-oral regimens were as good as or better than the then standard of care (72).

Objective 2: *Develop and implement a population pharmacokinetic and pharmacodynamic study of the investigational drugs used in the TB-PRACTECAL trial (PRACTECAL-PKPD).*

A one-compartment disposition model with first order absorption and elimination disposition model with fat-free mass allometric scaling and a Caucasian race on clearance best described the linezolid pharmacokinetics. The clearance was 5.88 L/hr, a volume of 58.5 L. At a daily dose of 600mg, the median $AUC_{0\text{-}24}$ was 90.40 mg*h/L and at 300 mg daily the median $AUC_{0\text{-}24}$ was 45.67 mg*h/L. The linezolid median MIC was 0.5 mg/L. The 600mg dose probability of $fAUC_{0\text{-}24}$ / MIC target of 119 was reached for MIC of 0.25 mg/L.

A one-compartment first order absorption and elimination model with allometric scaling of fat-free mass on both clearance and volume of distribution best characterised pretomanid pharmacokinetics. The clearance was estimated at 3.08 L/hr and a volume of 103L. Pretomanid median MIC was 0.125 mg/L. Virtually all patients in the TB-PRACTECAL trial (200mg daily) had drug exposures above 77%*f*T>MIC target and at least 96% would have been above the 167 $AUC_{0\text{-}24}$/MIC target.

A two-compartment first order absorption and elimination model with a lag time absorption parameter best described the pharmacokinetics of clofazimine. The only covariate identified was body weight allometry on clearance and intercompartmental clearance. The clearance was 6.84 L/hr and volumes of central and peripheral compartments were 1,750L and 9,150L respectively. The median MIC was 0.188mg/L. Using the 100mg daily flat dosing as in TB-PRACTECAL, the probability %T>MIC target could only be achieved up to an MIC of 0.5mg/L.

Bedaquiline population pharmacokinetics was best described by a three-compartment model with fixed transit compartments. BMI was the only covariate included in the final model. The clearance was 1.93 L/hr and the volumes were 102l, 6520l and 93.7l for central, first and second peripheral compartments respectively. When dosed at the standard 400mg daily for two weeks followed by 200mg three times a week, probability of target attainment above 90% is only achieved for MGIT MICs below 0.063mg/L.

8.2. Research in context

The safety and efficacy results from the TB-PRACTECAL trial are consistent with those from other trials investigating 6-months all oral regimens containing bedaquiline, pretomanid and linezolid for the treatment of drug-resistant TB (32, 135, 137). Recent interim data from programmatic settings have also reported similar findings (138).

The clearances and volumes of distribution estimated from the PRACTECAL-PKPD study patients are within the ranges reported for linezolid (75, 80, 83-87), pretomanid (49, 97-100), clofazimine (111, 112, 114) and bedaquiline (43, 44, 120). However, comparison of the PKPD target achievement results was limited by differences in mycobacteriology culture methods used for establishing MICs in published studies (49, 75, 86, 95, 109, 120).

8.3. Strengths

8.3.1. Technical strengths

TB-PRACTECAL's randomised and controlled design was one of its main strengths that facilitated its rapid adoption into global policy (32, 139). High efficacy results of the BPaL regimen in the NiX study (137) were not convincing to WHO to recommend it as a standard of care not only due to the concerns around safety of high linezolid doses but also due to the absence of randomisation and a control (31). Some experts even challenged the use of a single-arm study to approve pretomanid by the United States Food and Drug Authority (140).

A strength of the TB-PRACTECAL trial was that it was a multi-arm multistage (MAMS) clinical trial, which is a type of an adaptive trial design (141). This differs from traditional trial design in which aspects of the trial such as sample size, adding or dropping treatments, treatment allocation ratios or endpoints can be adapted during trial conduct. This additional 'review-adapt loop' as shown in figure 8.1, allows flexibility in case some assumptions are proven wrong and still provides an opportunity to successfully answer the research question (142). TB-PRACTECAL dropped the BPaL and BPaLC arms at the end of stage 1 as the main adaptation which facilitated the timely completion of the trial.

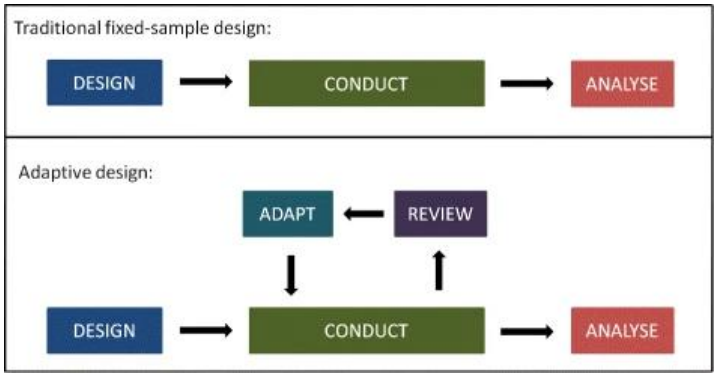


Figure 8.1: Fixed and adaptive clinical trial design. Adapted from Pallmann et al. 2018

TB-PRACTECAL was also implemented both as an operationally (i.e. no pause in recruitment between stages) and inferentially (i.e. utilise data collected from both stages for the final analysis) seamless design (143). This design approach reduced both the required sample size and time to achieve that sample size. MAMS designs have challenges intrinsic to their design, such as controlling for the family wise error rate due to multiple comparisons (144). We used the Bonferroni correction thus a one-sided type I error of 1·7% was assumed in the primary stage 2 analysis (145). Some other challenges are more practical and often arise from lack of understanding or willingness to accept adaptive designs; the transition from stage one to stage two was delayed as some ethics committees and regulators preferred to approve a protocol amendment resulting in a delay in trial completion hence reducing the efficiency of the sample size gains (146). One journal required as a condition to publishing the stage two results that the outcomes of the dropped arms should be integrated, delaying the publication for a significant period.

PRACTECAL-PKPD used the experimental design (ED) optimal design approach to ensure that the study design was efficient. Optimal design theory uses prior information (drugs popPK models and their parameter estimates) to optimize a function of the Fisher information matrix (FIM) to obtain the best combination of the design factors such as the timing of sample collection within the defined operational limitations (79, 147). Despite the maximum sample size not being reached, sparse sampling resulted in good data fitting and stable population pharmacokinetic models for all drugs except moxifloxacin (data not included in thesis) but this was expected from the results of the optimised sampling schedule evaluation (79).

In addition to TB-PRACTECAL's design and results, the addition of a sub-study that assessed the evolution of health-related quality of life (QoL) from baseline, throughout treatment, and after treatment completion in a subgroup of trial patients (PRACTECAL-PRO) (148) was key to global policy change. The PRACTECAL-EE, another sub-study of TB-PRACTECAL, aimed at assessing the costs to patients and providers of new regimens, as well as their cost-effectiveness and impact on participant poverty levels (149), a modelling analysis using interim data supported the preference of BPaLM in the WHO guidelines (32, 150).

8.3.2. Contextual strengths

TB-PRACTECAL recruited a cohort of patients that is quite representative of the global DR-TB population. Sites with high second line drug resistance (Belarus and Uzbekistan), sites with high proportions of patients living with HIV (South Africa) assured the diversity. Genetic diversity was not only contributed to by geography but sites also had individual of black and Caucasian race included, however there were very few participants with an Asian background.

Recognising that adherence to treatment is a significant driver of outcomes (151), support to study participants varied depending on the local practice i.e. prolonged hospitalisation with directly observed therapy (DOT) in Tashkent and Minsk, community DOT in South Africa and later due to the COVID pandemic video DOT was commonly used (152).

For the PRACTECAL-PKPD, the flexibility in adherence support can be considered as a strength as it is representative of how patients would take their treatment however it could also be a limitation as the timing of the drug intake is not always confirmed resulting in errors in sample time collection in relation to dose intake. This is however accounted for in the residual variability function in the statistical models (153).

The study had a significant component of community engagement where not only were the patients and community involved in trial implementation abut also in the design (154).

8.4. Limitations

Despite the clear value in inclusion of an internal standard of care control in the clinical trial, it posed several limitations. As the rifampicin resistant TB treatment standard of care varied according to national policy and improved over the years (155), the trial's control was inconsistent and resulted in concerns around indirectness of the final trial analysis. However, sensitivity analysis showed that the BPaLM effect estimate though smaller at −19·1% (95% CI: -30.9% to -7.3%) when participants recruited before the 2019 WHO drug resistant tuberculosis guidelines were implemented were excluded, it still showed superiority.

Being open label, there was a risk that there could have been an increased risk of bias and overestimation of beneficial effects (156). The laboratory personnel in the trial were however blinded to group allocation and therefore reducing bias on the efficacy outcomes.

The covid pandemic significantly disrupted clinical trials including by reducing or halting enrolment, inconsistent or halting of data collection (157, 158). TB-PRACTECAL was not an exception, to ensure continuity some amendments to the protocol were done including not requiring a face-to-face consultation for up to two weeks and using video DOT. A sub-group analysis showed that although the BPaLM efficacy risk difference reduced, it still significantly favoured BPaLM.

The interpretation of probability of target attainment was significantly limited by the targets being derived from studies that used different techniques to measure the MIC. There was no possibility to understand whether the MIC ranges from literature were different due to the PRACTECAL population being different or this was due to the difference in culturing methods. Since the differences could be only one dilution but this could have a significant different in interpretation. In addition, although there was a single manual and strict oversight, TB-PRACTECAL used three different mycobacteriology laboratories, and this could also have been a source of variability.

The estimated proportion of drug that is protein bound in plasma is 31%, 85-97%, 85-99% and >99.9% for linezolid (159), pretomanid (49, 95), clofazimine (109, 111), and

bedaquiline (133) respectively. As it is only the free, unbound drug that binds to a target to have any pharmacological effect, understanding protein binding is important. However, the limitations of adequately taking this in consideration for our study include the absence of within study free drug measurements and hence only utilising literature, the simplicity of our models to take into account the plethora of factors influencing the relationship between protein binding and PKPD (160) and finally absence of consideration to drug binding to culture media and plastics when estimating the MIC (161). Moreso, scaling total plasma concentration by free/unbound fraction (fu) to calculate the free plasma concentration carries over the higher variability of total plasma concentration into the PKPD index calculation thereby introducing bias (162).

In interpreting probability of target attainment results in our study, consideration must be made that drug exposure variabilities between plasma and other body tissues and within components of tuberculous granuloma may impact the overall drug efficacy (3, 163).

8.5. Policy implications

Data from the TB-PRACTECAL trial was used by the WHO 2022 guidelines development group (GDG) for the treatment of rifampicin resistant TB. The guideline then communicated that WHO suggests the use of the 6-month treatment regimen composed of bedaquiline, pretomanid, linezolid (600 mg) and moxifloxacin (BPaLM) rather than 9-month or longer (18-month) regimens in MDR/RR-TB patients(32).

The rapid adoption of BPaLM was facilitated by the favourable efficacy and safety results, the trial design that included a control arm, the early communication and open sharing of trial data with WHO and availability of preliminary results of the PRACTECAL-PRO and the PRACTECA-EE. Although the interim PK results from PRACTECAL-PKPD were presented to the GDG, the absence of PD analyses precluded the GDG from making any dosing decisions based on it.

The PRACTECAL trial results as well as specifically requested data has been presented and shared with the national TB programmes of: Belarus, South Africa, Uzbekistan, India, the US ATS/CDC/ERS/IDSA United States guidelines group, China CDC, TB-REACH

projects, and at the Union World conference on lung health, International AIDS conference, CROI and many other meetings and conferences.

8.6. Further research

Patients with rifampicin resistant tuberculosis may now have significantly better treatment options but there are still lingering explanatory questions.

8.6.1. Does the level of each drug's exposure affect outcomes?

A priority question is to investigate the contribution of each of bedaquiline, pretomanid, linezolid, moxifloxacin and clofazimine to the microbiological efficacy, clinical efficacy and safety outcomes in the TB-PRACTECAL trial. All the data for this analysis were collected in the TB-PRACTECAL trial and PKPD study and modelling work has started.

8.6.2. Are some people more prone to linezolid (oxazolidinone) toxicity?

As a continuation of the project objectives, we will conduct genome-wide association (GWAS) analyses, focused SNPs analysis of association for PRACTECAL-PKPD participants and further exploration of mitochondrial haplotypes associated with known linezolid adverse reactions.

8.6.3. Can less thermolabile sample types and easier sample collection methods improve access to PK measurements?

Recognising the importance of integrating pharmacokinetic studies in both phase 2 and phase 3 studies (164), as well as consideration of therapeutic drug monitoring in TB (165), exploration of methods that can facilitate this are studied in the PRACTECAL Hair where small hair samples are used to measure cumulative drug exposure and PRACTECAL VAMS which is aimed at determining the accuracy of anti-TB drugs quantification using volumetric absorptive microsampling.

8.6.4. Are BPaLM and BPaLC regimens effective and safe in the real world?

Real world experience understanding of the outcomes of the BPaLM and BPaLC is proving promising, use of next generation sequencing to help make quick decisions have been

implemented in Belarus and Uzbekistan and the results will inform programmatic implementation (138).

8.7. Conclusion

Rifampicin resistant tuberculosis remains a global scourge and cause of suffering at individual, family and community level. Of those developing the disease, too few are diagnosed and even fewer are put on optimal treatment (17). The TB-PRACTECAL trial offered a few pieces to the puzzle – short, effective, safe and tolerable treatment regimens. The WHO recommended the use of BPaLM for rifampicin resistant tuberculosis and BPaL in patients with additional quinolone resistance (pre-XDR-TB) (32). The strength of the evidence emanating from the design, conduct and analysis of the randomised controlled trial, as well as the supportive economic evaluation and patient reported outcomes studies facilitated rapid global uptake of these regimens. Results of the pharmacokinetic study offer reassurance that participants had adequate drug exposures, however further pharmacodynamic analyses are required to complete the picture.

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10. Appendices

TB-PRACTECAL
Informed Consent Form for PRACTECAL-PKPD study
South Africa / Belarus
Version 1.0 of 20th November 2018

Title of study: A randomised, controlled, open-label, phase II-III trial to evaluate the safety and efficacy of drug regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug resistant tuberculosis (TB-PRACTECAL) – Protocol Number: **NCT02589782**

Title of sub-study: PRACTECAL-pharmacokinetic pharmacodynamic (PRACTECAL-PKPD) study
The PRACTECAL-PKPD research project is a sub-study of the main TB-PRACTECAL trial, focused on the investigational regimens only.

Sponsor: Médecins Sans Frontières Holland

Principal Investigator name and contact details: [xxxxxxxxxx], E: [xxxxxxxxxx] Tel: [Doris Goodwin 033 3980054, Don McKenzie 031 7771009].

Participant's Printed Name: _____

This Informed Consent Form has three parts:

- Part I (Information Sheet) is to share information about the study with you, it tells you the purpose of this study and what will happen to you if you take part.
- Part II (Certificate of Consent) is for signatures if you agree to take part in the PKPD.
- Part III (Certificate of Consent) is for signatures if you agree to take part in the PG component.

You should keep this information sheet safe and refer to this throughout the study if you decide to take part.

Part I: Information Sheet

Dear Madam or Sir

This consent form may contain words that you do not understand. Please ask a study doctor or a member of the study staff to explain any words that you do not know, or any information that is unclear or confusing.

We would like to invite you to take part in our research study. Before you decide we would like you to understand the research and what it would involve for you.

PRACTECAL-PKPD is a sub-study of the main TB-PRACTECAL trial which you have agreed to participate in therefore all the commitments we made to you including reimbursement of transport and hospitalisation costs, freedom to stop taking part, protection of your privacy and compensation in the event of trial-related injury remain the same. Your participation in the various components of this sub-study is voluntary.

If you decide that you do not want to participate in any or all the components of this sub-study, you may still participate in the main TB-PRACTECAL clinical trial.

What is the sub-study about?

The study involves measuring the level of medicines in your blood and hair and looking at your genetic make-up.

You have been or may be allocated to an investigational arm of the TB-PRACTECAL trial that may include the following drugs: pretomanid, linezolid, bedaquiline, clofazimine and moxifloxacin. Although the individual drugs have been shown to work well, there isn't enough information known about the levels in the blood that these drugs reach when given together and how this is related to how well you do on treatment. We will therefore test the levels to understand these questions better.

We will also like to understand why some people have higher or lower levels of drugs in their blood and whether this is related to how well you do or how much side affects you get by looking at your genetic makeup specific to the way the body processes and gets rid of the drugs.

The collection and storage of the blood for measuring the drug levels is very difficult so we will also test methods of doing it differently including measuring the drug levels in small samples of hair and in very small amounts of blood.

What additional investigations will you be requested to undergo?

Most of the study visits and tests are at the same time points as for the main trial. However, you may be asked to spend the night in the hospital twice in the course of the study if you are not already hospitalised or don't live close to the hospital in order to have tests done early morning on the day of the sample collection (before drug intake), later in the day and early morning the day after (23 hours after drug intake).

What happens at the study visits?

- **Blood draw:** The study doctor or study nurse will draw about 4 ml of blood (one teaspoon) from your arm or hand. On two occasions there will be 3 blood collections within 24 hours adding up to about 12 ml of blood (one tablespoon). On one occasion we may take an additional 2ml (half a teaspoon) as well.

- **Finger prick:** A finger prick test to evaluate a new method of collecting very small amounts of blood will be done as well at each visit. The amount of this sample is less than a drop, it is the same method used for other tests (e.g. blood sugar test).

- **Hair sample**: a small thatch of hair is cut as close as possible at the scalp at the back part of the head at defined timepoints.

Visit Number	**3**		**7**	**8**	**9**	**10**	**11**	**12**	**17**
Timing of trial visit	**D1**	**D2**	**W 8**	**W 12**	**W 16**	**W 20**	**W 24**	**W 32**	**W72**
Medical history and Physical examination	x	x	x	X	x	x	x	x	x
Blood test	**x x x**	**x**	**x x x**	**X**	**x**	**x**	**x**	**x**	**x**
Hair sample			**x**		**x**		**x**	**x**	**x**

Table 1 PRACTECAL-PKPD sub-study investigational schedule

Where will my samples be tested?

The samples collected during the study will be shipped and analysed at the University of Liverpool, United Kingdom, University of California, San Francisco and Rutgers, The state university of Jersey in the United States of America. The samples will be destroyed after all laboratory analyses of the study have been completed.

How many people will be in this study?

The study will take place in the following TB-PRACTECAL trial sites:

1) Republican Scientific and Practical Centre of Pulmonology and Tuberculosis in Minsk, Republic of Belarus
2) Doris Goodwin Hospital, Pietermaritzburg, KwaZulu Natal, South Africa
3) Don McKenzie and King Dinuzulu Hospitals, KwaZulu Natal, South Africa
4) Helen Joseph Hospital, Gauteng, South Africa

There is no set number of people expected to participate in this research, we expect to involve up to 240 patients.

What are the risks and possible discomforts of being in this study?

Giving blood may be associated with discomfort and may leave a temporary bruise. Every effort will be made to minimise this. The amount of blood given at each visit is only a tiny fraction of the amount in your whole body so you should not feel any ill effects from this.

In case of need, a cannula may be inserted for multiple blood testing visit (to avoid three skin punctures), this may be associated with discomfort, occasionally can cause swelling, redness, heat, and pain, occlusion or infection.

What are the possible benefits of being in this study?

There is no guarantee that you will directly benefit from being in this study. The results of this study may guide the future treatment of MDR-TB. Others in the future may benefit from knowledge gained during this study by helping us find a shorter and more effective treatment for MDR TB.

What are your responsibilities if you participate?

If you agree to participate in the study, we expect you to comply with the study requirements and procedures.

What should you do if you want to stop taking part in the study?

You can decide to stop being in the study at any time, for any reason and you do not have to explain why. You can remain in the main trial if you want to and continue to receive the same treatment. If you decide to stop being in the main study, it will not affect the quality of your care, and you will still receive treatment for your MDR TB, according to the standard of care in use in [South Africa / Belarus]. Any information collected up to the point you stop cannot be removed from the study. This includes any samples collected up until the date of withdrawal, which will be kept and analysed. If you decide to stop participating, please inform your study doctor or nurse.

Who has reviewed the study?

All research studies are reviewed by an independent group of people, called a research ethics committee to protect your safety, rights, well-being and dignity. This study has been reviewed and approved by both [PharmaEthics] Research Ethics Committee and the [South African Health Products Regulatory Authority (SAHPRA) of South Africa] for compliance with medical and ethical standards. In addition, the study will be conducted according to the latest version of [DOH] (declaration of Helsinki) and Guidelines for Good Practice in the Conduct of Clinical Trials [with a Human Participant in South Africa, 2nd Edition 2006,] which deal with your rights as a research participant and guide the study doctor (investigator) in biomedical research involving human participants.

The proposal has also been reviewed and approved by the ethics committee of MSF, who is sponsoring the study.
The Sponsor, Regulatory Authorities or the Ethics Committee may stop the study at any time where there is good reason.

If you have questions or concerns about this study, whom can you call?

If you have questions, concerns, or believe you may have developed an injury related to this study, contact [xxxxxxxxxxxx] at [033 3980054] (Doris Goodwin), 031 7771009 (Don McKenzie).

For more information about the study, your rights, and in the event of a study related injury or side effect/adverse event, please contact:

Name of study personnel: _____

Phone (incl. area code): _____ Fax: _____

Study site Address:_____

Email (if applicable):_____

The 24-hour emergency phone number is: _____

Pharma-Ethics Research Ethics Committee
PO Box 786
Irene, 0062
Tel: (0) 12 664 8690
Fax: (0)12 664 7860
e-mail: marzelle@pharma-ethics.co.za

If you have questions about this trial, you should first discuss them with the study doctor or the abovementioned ethics committee. If you do not receive answers that are to your satisfaction, you should write to the National Health Research Ethics Council or the Medicines Control Council at:

The Chair

National Health Research Ethics Council

Tel: (012) 395 8113

Fax: (012) 3958467

E-mail: nhrec@health.gov.za

The Registrar

Medicines Control Council

Department of Health

Private Bag X828

Pretoria, 0001

Fax: (012) 3959201

Email: gouwsj@health.gov.za or mogobm@health.gov.za

It is important that you know that you have the right not to participate without giving a reason and without any penalty or loss of benefits.

Thank you for reading this and considering if you will take part in this study.

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

TB Practecal
Innovating MDR-TB Treatment

Part II: Certificate of Consent for PKPD Sub-Study

Title of study: A randomised, controlled, open-label, phase II-III trial to evaluate the safety and efficacy of drug regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug resistant tuberculosis (TB-PRACTECAL)

Title of sub-study: PRACTECAL-pharmacokinetic pharmacodynamic (PRACTECAL-PKPD) study
The PRACTECAL-PKPD research project is a sub-study of the main TB-PRACTECAL trial, focused on the investigational regimens only.

Before making the decision regarding participation in this study, I have discussed this study with an investigator and have been informed of the objectives, procedures, risks, and benefits of the study. I have had the opportunity to ask questions.

Participant:

By signing this consent form, I voluntarily choose to take part in:

- ☐ The main PKPD sub-study (blood draws)
- ☐ The hair sub-study (hair collection)
- ☐ The microsampling sub-study (finger prick)

_____ _____

Signature of Participant Date (dd/mmm/yyyy)

_____

Printed Name

Witness (if participant is illiterate):

I have witnessed the accurate reading of the consent form to the participant. I confirm that the individual has had the opportunity to ask questions. I confirm that the individual agrees to be part of the study.

_____ _____ AND Thumbprint of participant

Signature of Witness Date (dd/mmm/yyyy)

_____

Printed Name of witness

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

TB Practecal
Innovating MDR-TB Treatment

Part III: Certificate of Consent for genetic research

Title of study: A randomised, controlled, open-label, phase II-III trial to evaluate the safety and efficacy of drug regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug resistant tuberculosis (TB-PRACTECAL)

Title of sub-study: PRACTECAL-pharmacokinetic pharmacodynamic (PRACTECAL-PKPD) study
The PRACTECAL-PKPD research project is a sub-study of the main TB-PRACTECAL trial, focused on the investigational regimens only.

Before making the decision regarding participation in this component of the sub-study, I have discussed this study with an investigator and have been informed of the objectives, procedures, risks, and benefits. I have had the opportunity to ask questions.

Participant:

By signing this consent form, I voluntarily choose to take part in the pharmacogenomic (genetic) research:

_____ _____

Signature of Participant Date (dd/mmm/yyyy)

_____

Printed Name

Witness (if participant is illiterate):

I have witnessed the accurate reading of the consent form to the participant. I confirm that the individual has had the opportunity to ask questions. I confirm that the individual agrees to be part of the study.

_____ _____ AND Thumbprint of participant

Signature of Witness Date (dd/mmm/yyyy)

_____

Printed Name of witness

_____

Investigator:

I have explained the study to the subject, and answered all of his/her questions. I believe that he/she understands the information described in this document and freely consents to participate. A signed copy of this ICF has been provided to the participant.

_____ _____

Signature of investigator Date (dd/mmm/yyyy)

_____

Printed name of Investigator

_____

Printed name of person conducting consent (other than the investigator)

_____

Signature of person conducting consent (other than the investigator)

_____

Date (dd/mmm/yyyy)

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20 March 2019

HANDED TO

Ms C Van Maanen

IQVIA
PO Box 4407
TYGER VALLEY
7536

Fax: 0219147425

Dear Ms Van Maanen,

PROTOCOL: NCT02589782

A RANDOMISED, CONTROLLED, OPEN-LABEL, PHASE II-III TRIAL TO EVALUATE THE SAFETY AND EFFICACY OF REGIMENS CONTAINING BEDAQUILINE AND PRETOMANID FOR THE TREATMENT OF ADULT PATIENTS WITH PULMONARY MULTIDRUG RESISTANT TUBERCULOSIS (TB PRACTEAL)

ETHICS REFERENCE NO: 170316357

RE : NOTIFICATION OF ANNEX 3 DATED 19 JAN 2019 FOR ALREADY APPROVED PROTOCOL AMENDMENT 6.1

We acknowledge receipt of your letter dated 20 March 2019 with the following documentation pertaining to the above-captioned trial.

We acknowledge receipt of the TB-PRACTECAL protocol v6.1 Annex 3_PRACTECA-PKPD study_19JAN2019 clean for the above-mentioned study.

The above has been noted for the Ethics Committee information and records.

KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / SPONSOR / STUDY CO-ORDINATORS - WHERE APPLICABLE

Regards

[redacted]

MRS MARZELLE HASKINS
For and on behalf of Pharma-Ethics

Extract from the Meeting Minutes
of the Ethics Committee
at the State Institution Republican Scientific and Practical Center for Pulmonology and Tuberculosis

dated July 12, 2019

THE MEETING WAS ATTENDED BY:
T.M. Kritskaya – Chair
Z.I. Rogova – Secretary
Members:
L.V. Litzkevich
N.I. Kudlach
A.F. Belko
L.I. Metelitsa
V.P. Avchinko

AGENDA:

1. Consideration of the new version of the Protocol and other documents for the international multicenter clinical study under Protocol: TB-PRACTECAL (# NCT02589782) "*A Randomised, Controlled, Open-Label, Phase II-III Trial to Evaluate the Safety and Efficacy of Regimens Containing Bedaquiline and Pretomanid for the Treatment of Adult Patients With Pulmonary Multidrug Resistant Tuberculosis*".

Principal Investigator: V.V.Solodovnikova

The following documents were submitted:

No.	Document title and version	Date
1	Annex D	May 15, 2019
2	TB-PRACTECAL Protocol v6.2	April 23, 2019
3	Protocol Annex II v6.0	November 20, 2018
4	Protocol Annex III (PRACTECAL-PKPD sub-study) v6.1	January 16, 2019
5	PRACTECAL-PKPD sub-study CRF v0.3	November 27, 2019
6	Informed Consent Form (Stage I) v6.2	April 23, 2019
7	Informed Consent Form for PRACTECAL-PKPD sub-study v1.1	January 16, 2019
8	Patient Booklet Belarus v6.1	April 23, 2019
9	Patient Flipbook v6.1	April 23, 2019
10	Patient Info Sheet v4.0	December 04, 2018
11	Patient Film Script v6.0	January 21, 2019
12	Reference Safety Information v5.0	November 27, 2018
13	Pretomanid_IB_v17_Addendum	March 25, 2019
14	Pretomanid_IB_v17_extension_communication	March 25, 2019
15	LSHTM Research Ethics Committee Approval for Protocol v6.0 and associated documents	January 04, 2019

16	LSHTM Research Ethics Committee Approval for PKPD sub-study and associated documents	January 10, 2019
17	MSF Ethics Review Board Approval	January 29, 2019

REPORTERS:

Regarding item 1 of the Agenda: V.V. Solodovnikova reported on the new version of the Protocol and other documents for the international multicenter clinical study under Protocol: TB-PRACTECAL (# NCT02589782) "*A Randomised, Controlled, Open-Label, Phase II-III Trial to Evaluate the Safety and Efficacy of Regimens Containing Bedaquiline and Pretomanid for the Treatment of Adult Patients With Pulmonary Multidrug Resistant Tuberculosis*".

The following documents were reviewed:

No.	Document title and version	Date
1	Annex D	May 15, 2019
2	TB-PRACTECAL Protocol v6.2	April 23, 2019
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16	LSHTM Research Ethics Committee Approval for PKPD sub-study and associated documents	January 10, 2019
17	MSF Ethics Review Board Approval	January 29, 2019

RESOLVED:

On cl. 1 of Agenda:

to approve the new version of the Protocol, other above listed documents.

Voting result: "Pro" – 7, "Contra" – no. Special opinion – no.

RESOLUTION. The Ethics Committee approved the new version of the Protocol and other presented documents for the international multicenter clinical study under Protocol:

TB-PRACTECAL (# NCT02589782) "*A Randomised, Controlled, Open-Label, Phase II-III Trial to Evaluate the Safety and Efficacy of Regimens Containing Bedaquiline and Pretomanid for the Treatment of Adult Patients With Pulmonary Multidrug Resistant Tuberculosis*" at the State Institution Republican Scientific and Practical Center for Pulmonology and Tuberculosis, the documents submitted.

Chair	<signed>	T.M. Kritskaya
Secretary	<signed>	Z.I. Rogova

Выписка из протокола
заседания комитета по этике
ГУ «РНПЦ пульмонологии и фтизиатрии»
от 12.07. 2019г.

ПРИСУТСТВОВАЛИ:
Крицкая Т.М. –председатель
Рогова З.И. - секретарь
Члены:
Лицкевич Л.В.
Кудлач Н.И
Белько А.Ф.
Метелица Л.И.
Авчинко В.П.

ПОВЕСТКА ДНЯ:

1. Рассмотрение новой версии Протокола и других документов для проведения международного многопланового клинического исследования по протоколу: TB-PRACTECAL (№ NCT02589782) *«Рандомизированное, контролируемое, открытое исследование фазы II-III для оценки безопасности и эффективности режимов с использованием бедаквилина и претоманида в лечении взрослых пациентов с туберкулезом легких с множественной лекарственной устойчивостью».*

Главный исследователь: Солодовникова В.В.

Представлены документы:

№ п/п	Название документа и версия	Дата
1	Приложение Д	15 мая 2019 г.
2	Протокол исследования TB-PRACTECAL, ред. 6.2	23 апреля 2019 г.
3	Приложение II, вер. 6.0 к протоколу исследования TB-PRACTECAL	20 ноября 2018 г.
4	Приложение III, вер. 6.1 к протоколу исследования TB-PRACTECAL (подысследование PRACTECAL-PKPD)	16 января 2019 г.
5	ИРК для подысследования PRACTECAL-PKPD, ред.0.3	27 ноября 2018 г.
6	Форма информированного согласия (Этап 1), ред. 6.2	23 апреля 2019 г.
7	Форма информированного согласия на участие в подысследовании PRACTECAL-PKPD, ред. 1.1	16 января 2019 г.
8	Брошюра для пациентов, Беларусь, ред.6.1	23 апреля 2019 г.
9	Журнал для пациентов, ред.6.1	23 апреля 2019 г.
10	Информационный листок для пациентов, ред.4.0	04 декабря 2018 г.
11	Сценарий фильма для пациентов, ред.6.0	21 января 2019 г.
12	Справочная информация по безопасности, ред. 5.0	27 ноября 2018 г.
13	Брошюра исследователя по препарату претоманид_ред.17_дополнение	25 марта 2019 г.
14	Дополнение к брошюре исследователя по препарату претоманид-ред.17	25 марта 2019 г.
15	Утверждение протокола (ред.6.0) и сопроводительных документов Комитетом по исследовательской этике Лондонской школы гигиены и тропической медицины	04 января 2019 г.
16	Утверждение подысследования PKPD и сопроводительных документов Комитетом по исследовательской этике Лондонской школы гигиены и тропической медицины	10 января 2019 г.
17	Утверждение наблюдательного совета по этике MSF	29 января 2019 г.

ВЫСТУПИЛИ:

По п. 1 повестки дня: Солодовникова В.В. с докладом о новой версии протокола и других документов для проведения международного многопланового клинического

исследования по протоколу: TB-PRACTECAL (№ NCT02589782) *«Рандомизированное, контролируемое, открытое исследование фазы II-III для оценки безопасности и эффективности режимов с использованием бедаквилина и претоманида в лечении взрослых пациентов с туберкулезом легких с множественной лекарственной устойчивостью»*.

Рассмотрены:

№ п/п	Название документа и версия	Дата
1	Приложение Д	15 мая 2019 г.
2	Протокол исследования TB-PRACTECAL, ред. 6.2	23 апреля 2019 г.
3	Приложение II, вер. 6.0 к протоколу исследования TB-PRACTECAL	20 ноября 2018 г.
4	Приложение III, вер. 6.1 к протоколу исследования TB-PRACTECAL (подысследование PRACTECAL-PKPD)	16 января 2019 г.
5	ИРК для подысследования PRACTECAL-PKPD, ред.0.3	27 ноября 2018 г.
6	Форма информированного согласия (Этап 1), ред. 6.2	23 апреля 2019 г.
7	Форма информированного согласия на участие в подысследовании PRACTECAL-PKPD, ред. 1.1	16 января 2019 г.
8	Брошюра для пациентов, Беларусь, ред.6.1	23 апреля 2019 г.
9	Журнал для пациентов, ред.6.1	23 апреля 2019 г.
10	Информационный листок для пациентов, ред.4.0	04 декабря 2018 г.
11	Сценарий фильма для пациентов, ред.6.0	21 января 2019 г.
12	Справочная информация по безопасности, ред. 5.0	27 ноября 2018 г.
13	Брошюра исследователя по препарату претоманид_ред.17_дополнение	25 марта 2019 г.
14	Дополнение к брошюре исследователя по препарату претоманид-ред.17	25 марта 2019 г.
15	Утверждение протокола (ред.6.0) и сопроводительных документов Комитетом по исследовательской этике Лондонской школы гигиены и тропической медицины	04 января 2019 г.
16	Утверждение подысследования PKPD и сопроводительных документов Комитетом по исследовательской этике Лондонской школы гигиены и тропической медицины	10 января 2019 г.
17	Утверждение наблюдательного совета по этике MSF	29 января 2019 г.

РЕШИЛИ:
По п. 1 Повестки дня:
одобрить новую версию протокола, другие вышеперечисленные документы.

Результат голосования: «За» - 7 , «Против» - нет. Особое мнение - нет.

ЗАКЛЮЧЕНИЕ. Комитет по этике одобрил новую версию протокола и другие представленные документы для проведения международного многопланового клинического исследования по протоколу: TB-PRACTECAL (№ NCT02589782) *«Рандомизированное, контролируемое, открытое исследование фазы II-III для оценки безопасности и эффективности режимов с использованием бедаквилина и претоманида в лечении взрослых пациентов с туберкулезом легких с множественной лекарственной устойчивостью»* на базе ГУ «РНПЦ пульмонологии и фтизиатрии», представленные документы.

Председатель [redacted] Т.М. Крицкая

Секретарь З.И.Рогова

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Human Research Ethics Committee: (Medical)
FWA Registered No IRB 00001223

SECRETARIAT: Suite 189, Private Bag x2600, Houghton 2041, South Africa Tel: +27-11-274 9200 Fax: +27-11-274 9281

02 July 2019

EMAILED & COURIERED

Ms E Mojapelo

Regulatory Manager
Clinical HIV Research Unit
Helen Joseph Hospital, Themba Lethu Clinic
Perth Road, Westdene
2092

Fax: 011 482 2130

Dear Ms Mojapelo,

PROTOCOL: NCT02589782 - A RANDOMISED, CONTROLLED, OPEN-LABEL, PHASE II-III TRIAL TO EVALUATE THE SAFETY AND EFFICACY OF REGIMENS CONTAINING BEDAQUILINE AND PRETOMANID FOR THE TREATMENT OF ADULT PATIENTS WITH PULMONARY MULTIDRUG RESISTANT TUBERCULOSIS - SHORT TITLE: PRAGMATIC CLINICAL TRIAL FOR A MORE EFFECTIVE CONCISE AND LESS TOXIC MDR-TB TREATMENT REGIMEN(S) (TB-PRACTECAL)

ETHICS REFERENCE NO: 190106

RE : FINAL ETHICS APPROVAL

This is to certify that the above-mentioned trial has been approved by the University of the Witwatersrand, Human Research Ethics Committee (HREC), and the Protocol/Expert Reviewer. Date of Meeting where trial was reviewed: 25 January 2019.

The University of the Witwatersrand, Human Research Ethics Committee Approval Granted for the above mentioned study is valid for five years. Where required by Sponsor to have approval on a more frequent basis it remains the responsibility of the Sponsor and Investigator to apply for continuing review and approval, or for the duration of the Trial.

1. It is the responsibility of the Sponsor and Principal Investigator to ensure, where required, that relevant approvals are in place and compliance with the following is adhered to before a trial may begin:

- If trial is being conducted in Provincial Health facilities: Approval from the Hospital CEO / Clinic Manager / District Research Committee (whichever is applicable) be obtained.
- The study is submitted onto The National Health Research Database (NHRD).
- The relevant approvals are uploaded onto the NHRD system: Ethics Approval, SAHPRA Approval, Hospital CEO / Clinic Manager / District Research Committee Approval.

* A copy of the SAHPRA Approval and/or SAHPRA Notification letter must be submitted to the Ethics Secretariat Office for record purposes (IF SAHPRA APPROVAL / NOTIFICATION IS APPLICABLE).

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :

- Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (Refer to POL-IEC–001 and SOP-IEC–005, Item 3.4).
- Any data received during the trial which, may cast doubt on the validity of the continuation of the study.

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:
* The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:
* The University of the Witwatersrand, Human Research Ethics Committee requests that the SAHPRA Progress Reports be submitted twice a year either in March and September or six monthly from start of study to the HREC Secretariat Office - 011 274 9281 and a report of the final results, at the conclusion of the study. (IF APPLICABLE)

4. REIMBURSEMENT TO PATIENTS FOR TRANSPORT:
* The Human Research Ethics Committee: (Medical) is in agreement that reimbursement per visit is according to the South African Health Products Regulatory Authority and that reimbursement should be appropriate according to the situation.

5. TRANSPORT AND STORAGE OF BLOOD AND TISSUE SAMPLES IN SOUTH AFRICA:
* If blood specimens are to be stored for future analysis and is planned that such analysis will be done outside Wits, then the blood must be stored at a facility in South Africa agreed with the relevant IRB, with release of sub-samples only once projects have been approved by the local Research Ethics Committee applicable to where the analysis will be done as well as by the Wits Human Research Ethics Committee: (Medical).

6. GENETIC TESTING:
* The Human Research Ethics Committee: Medical; will not approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. GOOD CLINICAL PRACTICE:
* The South African Department of Health, South African Health Products Regulatory Authority requires Good Clinical Practice (GCP) Training for all Investigators in Clinical Trials, and that GCP training be renewed every three (3) years.

As yet, there are no National Guidelines for the content of GCP courses. Until these are available the Wits Human Research Ethics Committee (Medical) will note courses completed by Investigators without approval of the content of the individual courses.

8. THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

8.1 Ethics Approval Form signed by the Chairperson of the HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator(s) per fax: 011 274 9281 for our records (this is applicable with the initial Approval).

8.2 List of members present at the HREC meeting held as per INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

9. WE AWAIT YOUR RESPONSES AS REQUESTED: Ensure to have these documents forwarded at the earliest for the HREC records.

* SAHPRA Approval letter and/or letter of Notification before the above study may commence / or where an Amendment may be implemented (IF SAHPRA APPROVAL / NOTIFICATION IS APPLICABLE). It remains the responsibility of the Principal Investigator and/or Sponsor to ensure that the relevant approvals are in place.

* Copy of Independent Ethics Declaration Approval Form signed by the Principal Investigator. (this is applicable with the initial Approval).

* Kindly forward the above to the undersigned at fax: 011 274 9281 at your earliest convenience.

The above has been noted for the Ethics Committee information and records.

KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / SPONSOR / STUDY CO-ORDINATORS - WHERE APPLICABLE

Regards,

[redacted]

PROF CLEMENT PENNY

For and on behalf of the Human Research Ethics Committee: (Medical)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

Ethics Reference No.	**190106**	**Date of Meeting**	**25-Jan-2019**
		Recertification Due	**29 November 2019 (If applicable)**

Principal Investigators:	Dr NP Ngubane	**Investigators:**	Dr S Badal-Faesen
	Dr MS Rassool		Dr JA Bennet
			Dr FM Conradie
			Dr NM Mvuna
			Dr NH Mwelase
			Dr VR Parker
			Dr LK White

Protocol Title:	**A Randomised, Controlled, Open-Label, Phase II-III Trial To Evaluate The Safety And Efficacy of Regimens Containing Bedaquiline and Pretomanid For The Treatment Of Adult Patients with Pulmonary Multidrug Resistant Tuberculosis - Short Title: Pragmatic Clinical Trial for a more Effective Concise and Less toxic MDR-TB treatment regimen(s) (TB-PRACTECAL)**

	DOCUMENTS REVIEWED	**Tick As Appropriate**		**Yes**	**No**
Protocol Number	**NCT02589782**	**Date:**	20-Nov-2018		
Protocol	NCT02589782 Protocol, Version 6.0	**Date:**	20-Nov-2018	☑	☐
	Annex 3: PRACTECAL-PKPD Study Detail	**Date:**	20-Nov-2018	☑	☐
Investigator's Brochure	Reference Safety Information - Version: 5.0 - Dated: 27 Nov 2018			☑	☐
Subject Information/Consent Form	Patient Film Script - Version: 6.0 - Dated: 16 Nov 2018			☑	☐
	Patient Flipbook - Version: 2.1 - Dated: 11 Dec 2018			☑	☐
	Patient Info Sheet - Version: 2.1 - Dated: 11 Dec 2018			☑	☐
	Patient Info Sheet (Booklet) - Version: 2.1 - Dated: 11 Dec 2018			☑	☐
	Directly Observed Treatment Card - Version: 3 - Dated: 26 Nov 2018			☑	☐
	Informed Consent Form for STAGE 1 in South Africa (CHRU) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Parental Informed Consent Form for STAGE 1 in South Africa (CHRU) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Paediatric Assent Form for STAGE 1 in South Africa (CHRU) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Participant Informed Consent for Human Immunodeficiency Virus (HIV) Testing (CHRU) - Version: 6.1 - Dated: 11 Jan 2019			☑	☐
	Parental Informed Consent for Human Immunodeficiency Virus (HIV) Testing (CHRU) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Paediatric Assent Form for Human Immunodeficiency Virus (HIV) Testing (CHRU) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Informed Consent Form for STAGE 1 in South Africa (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Parental Informed Consent Form for STAGE 1 in South Africa (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Paediatric Assent Form for STAGE 1 in South Africa (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Participant Informed Consent for Human Immunodeficiency Virus (HIV) Testing (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Parental Informed Consent for Human Immunodeficiency Virus (HIV) Testing (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Paediatric Assent Form for Human Immunodeficiency Virus (HIV) Testing (CHRU, King DinuZulu) - Version: 6.1 - Dated: 11 Feb 2019			☑	☐
	Informed Consent Form for pharmacogenomic study (PRACTECAL PG Sub-Study) (CHRU, King DinuZulu) - Version: 1.0 - Dated: 11 Feb 2019			☑	☐
	Informed Consent Form for pharmacogenomic study (PRACTECAL PG Sub-Study) (CHRU) - Version: 1.0 - Dated: 11 Feb 2019			☑	☐
Advertisements					
Questionnaires					
Insurance/Compensation	HDI - Certificate of Insurance - Policy No.: 01475683-14003	Valid From	01 May 2017 To: 31 Mar 2021	☑	☐
Synopsis of Study/Trial Summary	Pulmonary Multidrug Resistant Tuberculosis			☑	☐
Other Documentation	Protocol Synopsis - Dated: 20 Nov 2018			☑	☐
	HREC Trial Application - Dated: 07 Jan 2019			☑	☐
	PRA Trial Application - Dated: 07 Jan 2019			☑	☐
	NHREC Trial Application ID#: 4672 - Dated: 03 Feb 2017			☑	☐
	Research Collaboration Agreement - Dated:			☑	☐
	TB PRACTICAL - Budget (01 Jan 2019 - 31 Dec 22) - Dated:			☑	☐
	Justification of Placebo Arm - Dated:			☑	☐

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

	Annex II - Scientific Advisory Committee members - Dated: 20 Nov 2018	☑	☐
	MCC Approval Letter - Dated: 09 Jun 2017	☑	☐
Relevant Trial Hospital/(s)	King DinuZulu Hospital Complex ,Wits Health Consortium, CHRU	☑	☐
	Helen Joseph Hospital	☑	☐
Syndicate and/or Research Unit	King DinuZulu Hospital Complex ,Wits Health Consortium, CHRU	☑	☐
	Clinical HIV Research Unit, Wits - W CHRU	☑	☐

DETAILS OF COMMITTEE	
Name	University of the Witwatersrand Human Research Ethics Committee: (Medical)
Address	Research Office, Senate House University of the Witwatersrand, 1 Jan Smuts Avenue, BRAAMFONTEIN, Johannesburg, 2000

DETAILS OF MEETING	Yes	No
Is the Investigator a member of the committee ?	☐	☑
If "Yes" did he/she vote ?	☐	☑
Is the Committee organised and operated according to applicable laws and regulations together with ?	☑	☐
Local GCP requirements ?	☑	☐
ICH GCP requirements ?	☑	☐
FDA GCP requirements ? FWA Registered No. IRB00001223	☑	☐
Progress reports required either in March and September or six monthly from start of study ?	☑	☐

DECISION ON APPROVAL : Is approval given to conduct the trial ?		Tick As Appropriate
Yes - with no conditions		☑
Remarks :	Our expectation is that our requirements are fulfulled once a company has submitted and uploaded relevant approvals onto NHRD.	
Yes - with conditions		☐
Specify conditions :		
No		☐
Specify reasons		

SIGNATURES		
I confirm that the details on this form are correct:		Date
Name: **Prof C Penny** **Chair / Deputy Chair of Committee**	**Signature:** [redacted]	**02 July 2019**

DECLARATION OF INVESTIGATOR/(S)

To be completed and ONE COPY returned to the Secretariat for the HREC at Wits Health Consortium, 31 Princess of Wales Terrace, Parktown, 2193 or Fax To: 011 274-9281

I/We fully understand the conditions under which I am/we are authorised to carry out and complete the above-mentioned research and I/we agree to ensure full compliance with these conditions. Should any amendment, alteration or departure be contemplated from the research procedure methodology or manner of execution, I/we will communicate with the Chairman of the Human Research Ethics Committee: (Medical) for approval prior to acting on any of the above mentioned proposed amendments, alterations or departures. I am/we are fully aware that any unauthosised amendment, alteration or departure as above will amount to misconduct and may lead to the institution of disciplinary procedures.

Any approval given by the HREC is conditional upon consent being obtained by the Investigator/s from the Superintendent (or equivalent official) of the Hospital, Clinic or Institution in which the research is, in part or full, to take place.

The Chairman may of course at his discretion place the matter before the full Committee.

DATE:_____ SIGNATURE:_____ NAME:_____

PROTOCOL NUMBER **NCT02589782** ETHICS REF.: **190106**

Date Printed 02 July 2019

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE MEMBERS: (MEDICAL)
UNIVERSITY OF THE WITWATERSRAND

Attendance Register for the Ethics Meeting held on 25 January 2019 from 12:00 - 15:00

Venue: EXECUTIVE COMMITTEE ROOM, Ground Floor, Phillip V Tobias Building, Cnr York Road & 29 Princess of Wales Terrace

AFFILIATED TO THE UNIVERSITY OF THE WITWATERSRAND

Surname	Initials	Title	Discipline/s	Academic Qualifications	Gender	Present
Adam	Y	Prof	Obstetrics & Gynaecology	MB BCh; FCOG	F	Present
Conradie	FM	Dr	Infectious Diseases/HIV/TB	MB BCh; DTM&H; MSc; Dip HIV Man	F	Absent
Cooper	PA	Prof	Paediatrics	MB BCh, PhD, DCH (SA), FCPaeds (SA)	M	Present
Dhai	A	Prof	Biomedical Ethics	MB ChB; FCOG; LLM; PGDipIntResEthics	F	Absent
Donde	B	Prof	Radiation Oncology	MB BCh, MMed Rad (T)	M	Present
Etheredge	H	Dr	Biomedical Ethics	MSc Med, BA; PhD	F	Absent
Feldman	C	Prof	Pulmonology	MB BCh, PhD, DSc, FCP (SA); FRCP	M	Present
Gerrand	P	Dr	Social Work	PhD (Social Work)	F	Absent
Lownie	MA	Prof	Maxillo-Facial & Oral Surgery	BDS, BA (Hons), DipMFOS, FCMFOS(SA), MEd	F	Absent
Menezes	CN	Prof	Internal Medicine	MD, MMed (Int Med), Dip HIV Mang (SA), DTM&H, FCP (SA), Cert ID (SA), PhD	M	Absent
Naidoo	S	Prof	Public Health	MB BCh, DMTH, DHSM, DOH, MMED	M	Absent
Naran	NH	Dr	Chemical Pathology	PhD	M	Present
Penny	C	Prof	Internal Medicine	BSc Hons, PhD	M	Present
Ross	M	Prof	Public Health	MB ChB, FFCH(SA), FOM(UK)	F	Present
Sanne	IM	Prof	Infectious Diseases/HIV/TB	MB BCh, FCP (SA), DTM&H; MMed & PhD	M	Absent
Smith	C	Prof	Psychiatry	BA, BA (Hons), M.A (Clin.Psych), PhD	F	Present
Stewart	A	Prof	Physiotherapy	BSc (Physio), MSc, PhD, DPE	F	Present
Szabo	CP	Prof	Psychiatry	MB BCh, MMed, MScMed, PhD; FCPsych(SA)	M	Present
Thom	RGM	Prof	Psychiatry	MB ChB, DCH, FCPsych, PhD	F	Present
Tsotsi	NM	Dr		BDS; MPH; MSc Med; PGDipInt ResEthics	F	Present
Velaphi	S	Prof	Paediatrics	MB BCh, FCPaeds, MMed, PhD	M	Present
Wadee	R	Dr	Anatomical Pathology	MBBCh, FCPath, MMed	F	Absent
Warria	A	Dr	Social Work	D Litt et Phil (SWK)	F	Present
Willem	P	Dr	Human Genetics	MD, PhD	F	Absent
Woodiwiss	AJ	Prof	Cardiovascular Pathophysiology	BSc Physiotherapy, BSc, MSc, PhD	F	Absent

NOT AFFILIATED TO THE UNIVERSITY OF THE WITWATERSRAND

Surname	Initials	Title	Discipline/s	Academic Qualifications	Gender	Present
Barnabas	N	Ms	Civil Society Liaison Officer	Community Liaison Manager	F	Present
Burns	I	Mr	Community Representative	MA (Hons)		Present
Egan	A	Father	Theology	BA (Hons), MA, MDiv, STL, PhD	M	Absent
Guidozzi	Y	Adv	Lawyer	BSc (Nurs), LLB, MBA	F	Absent
Ikalafeng	B	Dr	Governance	BSc (Hons), MSc, PhD	F	Present
Langley	G	Dr	Nursing	MSc (Nursing), PhD, MPhil (Ethics); BA (Hons) Theol	F	Present
Mokhachane	M	Dr	Clinical Medicine	MB BCh, FCP (Paeds) SA, MMed, Neonatology (SA)	F	Present
Paruk	F	Prof	Anaesthesia	MB ChB, FCOG(SA), Crit Care(SA), PhD	F	Absent

Peter	JR	Adv	Lawyer	BCom; LLB; LLM	M	Absent
Van Gelderen	CJ	Prof	Obstetrics & Gynaecology	MB BCh, FRCOG, FCOG(SA)	M	Absent

RETIRED MEMBER OCCASIONALLY CO-OPTED FOR SURGICAL OPINION ON A PROJECT

Oettle	GJ	Prof	Surgery	BSc (Hons), MB BCh, FRCS	M

Note 1: This committee has been in continuous operation since October 1966

Note 2: The large committee size is to ensure a good attendance at meetings

Note 3: A Quorum is 12 members according to the 33% of members on a committee with more than 15 members as required by SA National Guidelines (ref 2 below)

This is to certify that the Human Research Ethics Committee: (Medical) of the University of the Witwatersrand operates according to the following guidelines of good clinical practice:

1. **ICH Harmonised Tripartite Guideline for Good Clinical Practice.**
2. **SA National Department of Health 2006 Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2006).**
3. **Declaration of Helsinki 2013.**

The Committee's United States Federal Wide Assurance details are:

1. **Country code SF.**
2. **FWA Number: FWA00000715.**
3. **University of the Witwatersrand: IORG0000862.**
4. **Human Research Ethics Committee: (Medical): IRB00001223.**

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Observational / Interventions Research Ethics Committee

Dr Bern-Thomas Nyang'wa
LSHTM

10 January 2019

Dear Bern-Thomas,

Study Title: PRACTECAL-PKPD

LSHTM ethics ref: 16249

Thank you for your application for the above research, which has now been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	TB-PRACTECAL protocol Annex 3_PRACTECAL-PKPD study_20NOV2018	20/11/2018	6.0
Information Sheet	PRACTECAL-PKPD_ICF SA model_v1.0_20Nov2018	20/11/2018	1.0
Information Sheet	PRACTECAL-PKPD_ICF_parental consent_SA model_V1.0_20Nov2018	20/11/2018	1.0
Protocol / Proposal	PRACTECAL-PKPD_CRF_V0.3 of 27Nov2018	27/11/2018	0.3
Investigator CV	NyangwaCV_Oct18	30/11/2018	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

The Union

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Virtual Event November 8-11

Linezolid exposure and Probability of Target Attainment in the TB-PRACTECAL clinical trial

E-Poster No. 1705

TBSCIENCE 2022

B-T. Nyang'wa[1-3], F. Kloprogge[2], I. Motta[1], A. Bustinduy[3], T. Apanasevich[4], R. Moodliar[5], D. Moore[3], G. Davies[6]
1 Médecins sans Frontières, UK & Netherlands; 2 University College London, Institute for Global Health;3 London School of Hygiene & Tropical Medicine; 4 RSPCPT, Belarus; 5 THINK, South Africa; 6 University of Liverpool, UK

Introduction

WHO recently recommended treatment of drug-resistant tuberculosis with linezolid (Lzd)-containing six month all-oral short regimens (BPaLM and BPaL). However, evidence on the optimal Lzd dose and duration is limited (a). **We aimed to estimate the population exposure metrics for Lzd given daily at 600 mg for 16 weeks decreased to 300 mg for 8 weeks.**

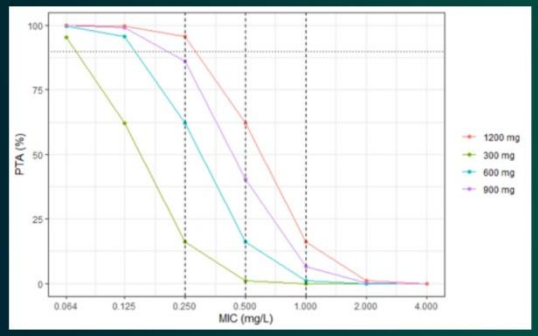
Methods

- MDR-TB patients from South Africa and Belarus participating in the TB-PRACTECAL Clinical Trial receiving bedaquiline, pretomanid and Lzd +/- moxifloxacin or clofazimine were recruited(b).
- Twelve samples at day 0, week 8 and up to 6 monthly troughs were collected.
- Lzd was measured using UHPLC-MS/MS.
- Data were analysed using nlmixr (2.0.4) R-package.
- Probability of Target Attainment (PTA) of fAUC/MIC >119 for 300mg, 600mg, 900mg and 1200mg doses were investigated for MIC range of 0.064 to 4.000 mg/L using 2,000 stochastic simulations.

Results

- 59 participants (62.7% males) with median BMI 20.6 kg/m contributed 553 drug measurements for analyses.
- Median of the Lzd trough concentrations was 331.38 (range 80–9991.4) ng/mL.
- A one-compartment model with transit absorption (N=6) and bodyweight allometry best described the data. Median MIC of 0.5mg/L (range 0.25–1), using Uzbekistan study data as reference. **PTA was below 80% for all investigated doses for median MIC .**

Linezolid exposure at all simulated doses **rarely reaches** the **currently defined PKPD target** despite the regimens being highly efficacious.

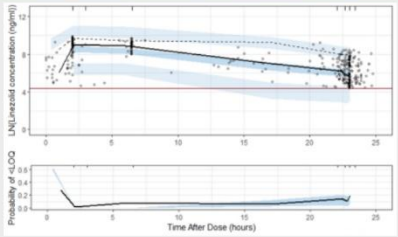


PTA (%) for Lzd at different doses at different MICs

Conclusion

- Despite TB-PRACTECAL regimens showing high efficacy, Lzd PTA was low, aligned with previous reports.(c,d)
- Further exploration of Lzd PK/PD targets when used in combination with bedaquiline and pretomanid is merited.

Tables and figures



Lzd pop PK model performance

	Estimate (95% CI)	Relative standard error	Between subject variability	Shrinkage
Estimated clearance (L/hr)	11.2 (9.1 - 13.8)	4.4%	59.2%	<18.7%
Estimated volume of distribution (L)	96.4 (70.8 - 131)	3.5%	81.5%	23.1%
Model transit time	1.61 (1.31 - 1.97)	21.7%		
Number of transit compartments	6 (fixed)			
Additive error	1.05			

Lzd pop PK model parameters

References

a. World Health Organization; 2022 (WHO/UCN/TB/2022.2).
b. Nyang'wa B-T. BMJ Open. 2021 Sep 6;11(9):e047185. doi: 10.1136/bmjopen-2020-047185.
c. Srivastava S, Antimicrob Agents Chemother. 2017;61(8):1-12. d Tietjen AT Br J Clin Pharmacol. 2022;88:1835-1844.

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TBScience 2021 Late-Breaker Abstract Submissions

Preview

Reference: AS-TBS-2021-01748

Title: PRACTECAL-VAMS: a successful novel approach to microsampling to determine TB drugs levels

[1]Center for Discovery and Innovation-Hackensack Meridian Health, Hackensack, United States of America, [2]Medecins Sans Frontieres, London, United Kingdom of Great Britain and Northern Ireland, [3]THINK, Tuberculosis&HIV Investigative Network, Durban, South Africa

Type selection

Submission Type: * Oral presentation

Theme selection

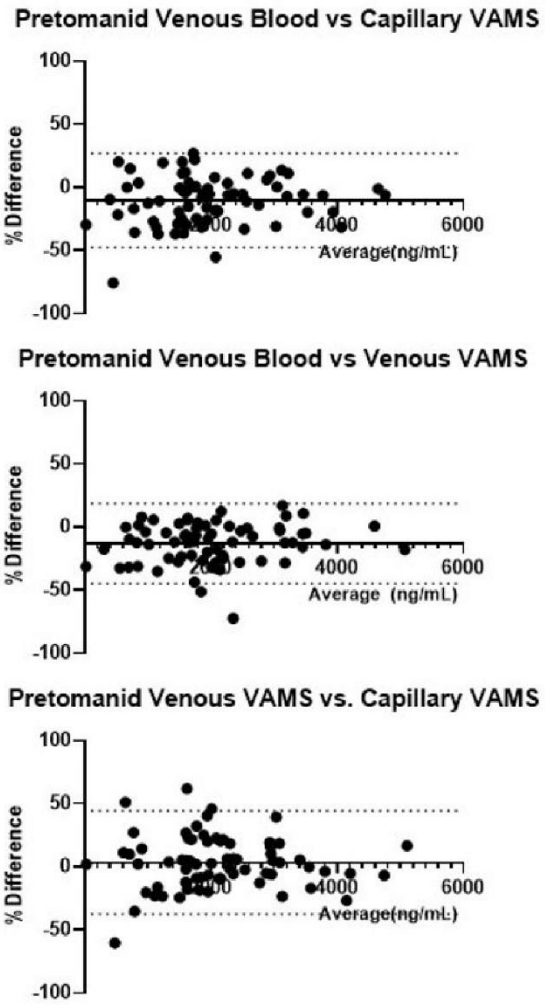
Theme: * THEME1: Tools to guide personalised therapy - what is achievable?

Abstract Text

Text: *

VAMS (volumetric absorptive microsampling) is a method used to collect, store and transport blood samples for measurement of drug levels. It is cheaper and easier to use, especially in resource constrained settings. The study was carried out in South Africa in a subgroup of participants enrolled in TB-PRACTECAL Clinical Trial's investigational arms containing bedaquiline (Bdq), pretomanid (Pa), linezolid (Lzd), moxifloxacin (Mfx) or clofazimine (Cfz). We aimed to determine the accuracy of quantification of anti-TB drugs using VAMS dried blood (capillary and venous) compared to liquid whole blood in cryotubes. Intensive PK samples at day 1 and week 8, and sparse samples at week 12 and 16 were collected. Drugs were extracted from blood samples by protein precipitation using organic solvents. Blood samples were quantified by HPLC-MS/MS. Data were analysed with STATA v.15 and Prism v.9.1.2. Preliminary data on Pa, Lzd and Mfx are presented.
Thirteen patients contributed 650 drug measurements for analysis. Correlation across all timepoints for Pa was 95.4% for VAMS capillary blood versus VAMS venous blood, 96.5% for VAMS capillary blood versus liquid venous blood and 96.3% for VAMS venous blood versus liquid venous blood. For Lzd it was 98%, 98.1% and 96.9%, respectively. For Mfx it was 96.5%, 95.8% and 99.3%, respectively. Figure 1 presents the Bland-Altman plots for Pa comparing the paired percentage difference of the three sampling techniques.
VAMS method results correlate highly with liquid blood. The results allow expanded access to blood level measurements for novel TB drugs. To our knowledge, this is the first time that data on Pa and Mfx sampled by VAMS are reported. We acknowledge the limited sample size, further analysis on Bdq and Cfz are pending and modelling for estimating exposure will be conducted.

Figure 1 Bland-Altman plots of pretomanid sampling techniques' comparison



1. I confirm that I previewed this abstract and that all information is correct: *	Yes
2. The corresponding author is responsible for informing the other authors: *	Yes

Back