Optimising linezolid use for drug-resistant tuberculosis: pharmacokinetics, toxicity, and resistance

SEAN ADAM WASSERMAN

MBChB, MMed, FCP(SA), CertID(Phys)SA

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

Department of Medicine

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

December 2021

Supervisors:

Professor Graeme Meintjes

Professor Gary Maartens

Table of Contents

DECLARATIONS

I, Sean Wasserman, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications:

1. Wasserman S, Meintjes G, Maartens G. Linezolid in the treatment of drug-resistant tuberculosis: the challenge of its narrow therapeutic index. Expert Review of Anti-infective Therapy. 2016 Oct;14(10):901-15.

   (Chapter 2 of thesis)

2. Wasserman S, Denti P, Brust JCM, Abdelwahab M, Hlungulu S, Wiesner L, Norman J, Sirgel FA, Warren RM, Esmail A, Dheda K, Gandhi NR, Meintjes G, Maartens G. Linezolid pharmacokinetics in South African patients with drug resistant tuberculosis and a high prevalence of HIV co-infection. Antimicrobial Agents and Chemotherapy. 2019; 26;63(3):e02164-18.

   (Chapter 3 of thesis)

3. Wasserman S, Louw G, Ramangoaela L, Barber G, Hayes, Cindy, Vally Omar S, Maartens G, Barry Clifton, Song T, Meintjes G. Linezolid resistance in patients with drug-resistant tuberculosis and treatment failure in South Africa. Journal of Antimicrobial Chemotherapy. 2019; 74(8):2377-2384.

   (Chapter 5 of thesis)

Signature: _____ Date: _____

Student Name: _____ Student Number: _____

ABSTRACT

Background: Rifampicin-resistant tuberculosis (RR-TB) accounts for an expanding proportion of incident global TB cases and is a major barrier to global tuberculosis control. There is a need for more effective, safe, and well-tolerated drugs. Linezolid is a repurposed oxazolidinone antimicrobial with bactericidal activity against *M. tuberculosis*. Guidelines recommend linezolid as a preferred antituberculosis agent for RR-TB, and it is widely used in national programmes. The major drawback of linezolid is dose-related mitochondrial toxicity that may be treatment limiting. The incidence and risk factors for linezolid adverse events have not been systematically studied in TB programmes, particularly in populations from sub-Saharan Africa with high rates of HIV co-infection, which could increase the risk of toxicity. Exposure-response relationships for linezolid toxicity are also not well characterised. In addition, limited data exist on clinical associations and genotypic correlates of linezolid resistance in *M. tuberculosis*, needed to inform strategies for resistance testing. My thesis aimed to address these knowledge gaps to optimise use of this important agent in RR-TB.

Methods: We conducted a prospective observational cohort study among patients with RR-TB across three sites in South Africa to characterise the pharmacokinetics (PK) and clinical toxicity of linezolid in programmatic settings with high HIV prevalence. Participants were followed for up to 24 months after linezolid initiation. We did monthly screening for peripheral and optic neuropathy, and collected clinical samples for toxicity outcomes, drug concentrations, and mitochondrial DNA analysis. Intensive PK sampling was performed on a subgroup of participants. Drug exposure was described using non-compartmental analysis and mixed effects modelling was used to analyse toxicity outcomes. For the resistance aims, we did a separate retrospective cohort study of patients with RR-TB and linezolid-based treatment failure at two TB referral hospitals in South Africa. Clinical information was extracted and recovered isolates underwent linezolid minimum inhibitory concentration (MIC) testing and targeted sequencing of *rrl* and *rplC*.

Results: Among 30 participants enrolled in the intensive PK study, linezolid exposure was related to body weight and age, but not HIV positivity. The standard 600 mg dose achieved the PK efficacy target at wild type MIC values, but trough concentrations

were above the putative toxicity threshold in almost 60%. 151 participants, 63% HIV-positive, were enrolled in the prospective cohort. Premature discontinuation of linezolid for toxicity was common but grade 3 or 4 adverse events occurred in 22 (15%). Linezolid trough concentration, male sex, and age (but not HIV-positivity) were independently associated with a decrease in haemoglobin > 2 g/dL. Trough linezolid concentration of 2.5 mg/L or higher resulted in optimal model performance to describe changing haemoglobin and was strongly associated with treatment-emergent anaemia. Single nucleotide polymorphisms 2706A>G and 3010G>A in mitochondrial DNA were not associated with linezolid toxicity. Thirty-nine patients with linezolid-based treatment failure were identified in the retrospective cohort, 13 (33%) of whom had phenotypic or genotypic linezolid resistance after a median duration of 22 months linezolid therapy. All isolates with phenotypic resistance were associated with known resistance mutations, most frequently due to the T460C substitution in *rplC*.

Conclusions: Our PK analysis confirmed the narrow therapeutic index of linezolid and there was no effect of HIV on linezolid exposure. Severe adverse events were uncommon at the standard dose of 600 mg daily and HIV co-infection was not independently associated with linezolid toxicity. Linezolid trough concentration of 2.5 mg/L should be evaluated as a target for therapeutic drug monitoring as a strategy to reduce toxicity. Resistance occurred late and was predicted by a limited number of mutations. Screening for genotypic resistance should be considered for patients with a positive culture after 4 months of linezolid therapy. These findings support current linezolid dosing in TB programmes.

ACKNOWLEDGEMENTS

This work was partly funded through a Career Development Fellowship awarded to me by the European and Developing Countries Clinical Trials Partnership (Grant number CDF1018). Additional support was provided by Wellcome (Grant number 203135/Z/16/Z) and the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant number 64787). The parent studies were funded by the US National Institute of Allergy and Infectious Diseases, National Institutes of Health (R01AI114304) and the South African Medical Research Council.

I am extremely grateful to all study participants and clinical staff at Jose Pearson Hospital, Brooklyn Chest Hospital, and King Dinuzulu Hospital for generously contributing their time. These studies would not be possible without the hard work and dedication of our research nurses, Siphokazi Hlungulu, Liziwe Rwentela, Primrose Mpangase, and Nokuthula Maluleka; laboratory staff, Francisco Lakay and Fatima Abrahams; study coordinators, Lindsay Joseph and Alexandria Hahn; and Meagan McMaster and Celeste Worship for capturing data. I am especially grateful to Rene Goliath for project management and to Kathy Wood for administrative support. I also thank Aubrey Shoko at the Centre for Proteomic and Genomic Research for extracting and sequencing mitochondrial DNA.

I have been privileged to learn from and work with all scientific collaborators on these projects. A special thanks to James Brust and Neel Gandhi who provided mentorship and friendship over the past several years. I am indebted to Paolo Denti and Mahmoud Abdelwahab who patiently attempted to teach me pharmacometrics and are now valued collaborators. CIDRI-Africa has been an incubator for my development as a clinician-scientist; I am very grateful to Robert Wilkinson for his support and guidance. A special thank you to Marc Mendelson, my clinical mentor and teacher.

I have been incredibly fortunate to have Graeme Meintjes and Gary Maartens as supervisors and mentors. I strive to emulate their scientific rigor and creativity, and more importantly, their humanity.

My family, a group of powerful women, are my inspiration. Granny Doreen, Mom, Jessica, Zia, Jade, and Emma continue to teach me the most important lesson of all - how to be a Mensch.

This work is dedicated to Siphokazi. You brought joy, vitality, and compassion to our lives.

And all this science
I don't understand
It's just my job five days a week

(Rocket Man)

CHAPTER 1

Introduction

Context and rationale

Burden and treatment paradigm of tuberculosis

Tuberculosis (TB) has long been the leading cause of death from a single infectious agent (expected to be displaced to second position by COVID-19 in 2020). Almost 10 million people develop active TB disease every year worldwide with an estimated 1.5 million deaths [1]. Declines in incidence are too slow, at less than 1% per year, to achieve World Health Organization (WHO) EndTB targets [2]. This severe global public health threat continues despite the existence of effective first-line antituberculosis agents.

The recalcitrance of *M. tuberculosis* infection, affecting a substantial proportion of the global population, is related to its ability to persist and relapse, requiring long durations of chemotherapy to achieve long-term cure. Clinical investigations performed since discovery and introduction of the first antituberculosis drugs in 1946 established that treatment with combination therapy is required. An early trial of streptomycin monotherapy found that although patients experienced dramatic initial clinical and microbiological improvement, acquisition of resistance led to universal relapse and a similar mortality after 5 years to those who received no treatment [3, 4]. As other antituberculosis agents became available, subsequent trials showed that use of combination therapy eliminated drug resistance on treatment. However, use of early drugs such as streptomycin, para-aminosalicylic acid, and isoniazid required long durations of poorly tolerated therapy, up to 12 months, to avoid disease relapse [5].

TB treatment was revolutionised in the 1970s when pivotal trials demonstrated that the newly discovered antimicrobials, rifampicin and pyrazinamide, could reduce relapse rates when combined with streptomycin and isoniazid for much shorter periods of 6 months [5, 6]. Treatment shortening was possible because of rifampicin's action against *M. tuberculosis* populations in stationary-growth phase and sterilizing activity throughout therapy, and the ability of pyrazinamide to kill extracellular bacilli in the early replicative stages of infection. The current paradigm for TB therapy was thus

established: use of drug combinations with early bactericidal activity (EBA) to rapidly reduce bacillary load to avoid clinical progression, transmission, and resistance emergence early in therapy, followed by agents with efficacy in chronic infection to eradicate persistent mycobacterial populations to achieve sterilisation and relapse-free cure.

The ‘short-course’ regimen established almost 50 years ago comprising rifampicin, isoniazid, pyrazinamide, and ethambutol (substituted for streptomycin or thioacetazone to reduce toxicity and provide protection in case of isoniazid resistance) for 2 months followed by rifampicin and isoniazid for 4 months is still in use, with 85% treatment success in programmatic settings [1]. However, 6 months of daily dosing with multiple pills is burdensome, complicated by frequent hepatotoxicity and poor tolerability, and requires complex infrastructure for delivery [7-10]. Incomplete treatment is an inevitable consequence, leading to relapse and possibly contributing to acquired drug resistance [11, 12]. Transmission of drug-resistant strains is driving an epidemic of rifampicin-resistant TB (RR-TB) [13], which represents a growing proportion of the global TB burden and numbers are expected to increase over the next two decades [14]. Emergence of RR-TB has threatened use of short-course antituberculosis therapy, driving a reversion to prolonged and toxic treatment durations used in the 1940s, with a major impact on global TB control.

Poor outcomes of rifampicin-resistant TB with conventional second line therapy

Until as recently as 2018, ‘conventional’ treatment regimens recommended by WHO for RR-TB included a backbone of pyrazinamide, an injectable agent (kanamycin, amikacin, or capreomycin), and fluoroquinolones in combination with at least one other second-line agent (para-aminosalicylic acid, ethionamide, and cycloserine) for a treatment duration of over 18 months (Table 1) [15]. This standardised drug regimen was inadequate for many patients in the context of high rates of background resistance to pyrazinamide and ethambutol [16], poorly effective second-line agents, and delayed results of second-line drug susceptibility testing to fluoroquinolones; one study showed that the pre-2018 standard empiric regimen included fewer than four effective drugs in 47% of patients with a rifampicin resistant GeneXpert result [17]. Over half of patients with RR-TB experienced an adverse event on conventional RR-TB therapy, with 70% requiring a change in treatment [18], until recently with no alternatives. The overlapping

toxicity of antituberculosis drugs used for RR-TB and antiretroviral therapy (ART) further limited the use of these regimens, particularly in Southern Africa where the TB epidemic is driven largely by HIV and co-infection rates exceed 60% [1]. Because conventional therapy for RR-TB was more toxic [18, 19], less effective [19, 20], and much more costly [21, 22] than drug-susceptible TB, treatment success has been consistently lower than 60% [19], and prior to widespread introduction of new drugs, RR-TB resulted in a quarter million deaths annually [23-25]. On conventional regimens, RR-TB had a case fatality ratio over 2.5-fold higher than in drug-sensitive TB [26], and although there is large variability by setting and comorbidities, overall mortality remains 25% and is higher among patients with HIV [27, 28]. The worst outcomes are seen with fluoroquinolone-resistant TB which is estimated to occur in over 20% of RR-TB cases globally [29] and had an overall 2-year mortality approaching 50% in the pre-bedaquiline era [30]. In addition to other drawbacks, the use of conventional RR-TB drug regimens resulted in resistance amplification during therapy [31, 32], thereby worsening the problem of RR-TB.

There is a clear need for more effective, safe, and well-tolerated drugs to support shorter and injection-free treatment regimens, improve adherence, and interrupt the cycle of resistance amplification and transmission of RR-TB. Key strategies to achieve this include dose optimisation of approved drugs, clinical development of new and repurposed drugs, and design of novel combination regimens that are better tolerated and more effective at preventing transmission and relapse [33].

Table 1. Evolution and role of linezolid in RR-TB treatment guidelines.

Year	WHO classification	Treatment guidance	Linezolid guidance	Evidence for linezolid
1996	Classification system not yet implemented.	First WHO guideline on drug-resistant TB.	LZD not mentioned	NA
2000	Classification system not yet implemented.	Standardised regimen not yet established. Recommendations based on expert reviews [34]	LZD not mentioned	NA
2003	List of “essential antituberculosis drugs” introduced. Included	Concept of standardised	LZD not mentioned	NA

	rifampicin, isoniazid, pyrazinamide, streptomycin, ethambutol, thioacetazone	treatment regimen introduced.		
2006 and 2008	Group-based classification system introduced.	Include injectable, FQ, PZA, EMB, and ≥ 1 of PAS, Eto, Cs/Trd. Total treatment duration 18 months after culture conversion (including at least 6 months injectable agent).	LZD listed in Group 5 (with clofazimine, amoxicillin/clavulanate, clarithromycin). Not recommended for routine use in MDR-TB treatment; contribution to the efficacy of multidrug regimens unclear.	No evidence presented. Based on "assessment of available evidence and best practice by a large group of TB specialists."
2011	Group-based classification unchanged.	Include PZA, FQ, injectable, Eto, and Cs (or PAS). Treatment duration extended for 2 months (8 months injectable, 20 months total).	LZD listed in Group 5. Not included among the drugs making up the standard regimen.	Systematic review to summarise available evidence with GRADE review [20, 35]. LZD not analysed because of small number of cases treated.
2014	Group-based classification unchanged.	Initial phase of five drugs, including an injectable agent, for eight months. Oral agents continue for a minimum of 12 months, for a total minimum treatment of 20 months.	LZD listed in Group 5. Limited data on efficacy and/or long-term safety in the treatment of drug resistant TB. LZD considered one of the most effective Group 5 agents and a key drug in XDR treatment regimens.	*In vitro* and animal studies. Cited observational clinical studies and Korean RCT for XDR-TB.
2016	Regrouping into 4 categories based on effectiveness in IPD-MA (mainly observational studies) [36, 37] and study-level meta-analysis.	Standardised shorter (9 - 12 month) MDR-TB regimen, still including an injectable agent, recommended under specific conditions. Conventional regimen included PZA plus four core second line drugs (FQ, injectable, plus either LZD, CFZ, Eto, or Cs) for 20 months.	LZD classified as core second line agent (Group C). Close monitoring for toxicity advised; if not possible, linezolid reserved for MDR-TB patients with additional drug resistance or who are intolerant to other components of the core regimen.	Cited same evidence as previous guideline. Data from only 39 patients included in the IPD-MA; numbers too small for definitive guidance.
2018	Regrouped into three categories and ranked based on evidence from an updated IPD-MA [38].	More individualized approach to treatment. Standardised shorter regimen unchanged	LZD designated Group A; recommended for inclusion in longer MDR-TB regimens unless contraindicated.	LZD use associated with improved treatment outcome and reduced mortality in IPD-MA.

		(including injectable) based on STREAM trial and observational data [39, 40].		
2019	Unchanged drug classification.	Individualised longer regimen including all Group A agents (BDQ, FQ, LZD) plus ≥ 1 Group B agent (CFZ, Cs/Trd) to make up ≥ 4. active agents. Total treatment duration of 18-20 months. Standardised shorter regimen unchanged (includes injectable)	LZD Group A drug; strong recommendation for inclusion in longer MDR-TB regimens. Suggested use for at least 6 months.	Same evidence as 2018 guideline.
2021 update	Unchanged drug classification.	Shorter all-oral regimen recommended for certain patients (BDQ substituted for injectable); does not include LZD.	LZD Group A drug; strong recommendation for inclusion in longer MDR-TB regimens. Conditional recommendation for use with BDQ/PMD (BPaL) for patients with MDR/RR-TB and additional FQ resistance only under operational research conditions.	Same evidence as 2018 guideline plus Nix-TB data [41].

Table 1. BDQ = bedaquiline, CFZ = clofazimine, FQ = fluoroquinolone, LZD = linezolid, PZA = pyrazinamide; IPD-MA = individual patient data meta-analysis.

Repurposed drugs enable treatment shortening

Repurposing existing antimicrobials for TB therapy is an attractive approach to fast-track drug development and address the urgent need for better regimens. Discovery of repurposed agents with potent EBA and/or sterilising potential has changed the treatment landscape for RR-TB. Fluoroquinolones are the earliest example, deployed as core drugs for RR-TB since the late 1990s, and recently shown to enable treatment shortening to 4-months in drug-susceptible TB [42].

Another repurposed drug, clofazimine, has emerged as a cornerstone of contemporary RR-TB regimens. First discovered in 1957, this riminophenazine antibiotic was used almost exclusively in combination therapy for leprosy but began entering small observational studies for TB in the early 2000s and was first listed as a so-called ‘Group

5' agent in WHO treatment guidelines in 2006. The breakthrough for clofazimine in TB therapy came after publication of an observational study in Bangladesh which demonstrated improved outcomes for TB in a programmatic setting with a shorter 9-month treatment regimen that included clofazimine [43]. In 2016, WHO issued a conditional endorsement of a standardised shorter (9 - 11 month) clofazimine-containing regimen for RR-TB [44], based largely on observational studies in Bangladesh [43, 45] and West Africa [46, 47]. Definitive evidence for shorter therapy in RR-TB was provided by the STREAM-1 trial, the first ever phase 3 randomised controlled trial (RCT) for a RR-TB regimen, which demonstrated non-inferiority of a standardised shorter regimen (containing high dose moxifloxacin, clofazimine, ethambutol, and pyrazinamide administered over a 40-week period, supplemented by kanamycin, isoniazid, and prothionamide in the first 16 weeks) compared with conventional 20-month therapy [48]. An individual patient data meta-analysis found that the inclusion of clofazimine in treatment regimens for RR-TB resulted in a significantly increased odds of treatment success and survival [38]. These observations are supported by mouse models which consistently demonstrate treatment-shortening potential and sterilizing ability of clofazimine [49, 50], which is now classified as a core agent for RR-TB by WHO and is currently being evaluated in several clinical trials for RR-TB (Table 2).

Bedaquiline and the transformation of RR-TB treatment

Although introduction of shorter therapy was a major advance in RR-TB therapy, the standardized regimen still contained an injectable agent and required treatment durations of up to 12 months. Perhaps the most important contemporary advance in TB therapy has been the discovery and accelerated FDA approval of bedaquiline in 2012. A diarylquinoline, bedaquiline has a unique mechanism of action by inhibiting mycobacterial ATP synthesis [51, 52], and enables treatment shortening [53-55] due to potent antimycobacterial activity [56]. Clinical trials and programmatic data have shown that inclusion of bedaquiline in RR-TB regimens results in improved treatment outcomes [57-59], including survival [60]. As a result, WHO recommended bedaquiline as a preferred agent for RR-TB therapy in 2018 [61], replacing injectables in longer treatment regimens. Given the evidence of improved outcomes with the addition of bedaquiline to conventional RR-TB regimens, and the favourable performance of shorter injectable-containing regimens, the South African National Department of Health departed from

WHO guidelines and implemented a 9-month regimen substituting bedaquiline for an injectable for the first 6 months of therapy. Use of an all-oral bedaquiline-containing shorter regimen was associated with higher treatment success rates (73%) compared with the standardized injectable-containing shorter regimen (60%) in programmatic data from South Africa [62]. Based on this, plus results from the Nix-TB trial, where bedaquiline (together with linezolid and pretomanid) enabled treatment shortening for fluoroquinolone-resistant TB [41], WHO made a conditional recommendation for use of an all-oral standardised shorter (9 - 12 month) regimen for RR-TB in 2019, revolutionising RR-TB treatment.

Linezolid for RR-TB

In parallel with the clinical development and implementation of clofazimine and bedaquiline in TB programmes, another repurposed agent, linezolid, was increasingly recognised as an important component of RR-TB regimens. As summarised in Table 1, linezolid first entered WHO guidelines as a drug of last resort in 2006, but quickly rose to prominence after 2012 when a landmark trial in Korea demonstrated its ability to achieve cure in patients with refractory extensively drug resistant (XDR)-TB [63, 64]. Linezolid is now recommended by WHO in longer regimens for RR-TB and is a component of several novel shorter RR-TB regimens under investigation (Table 2).

Table 2. Current Phase 2/3 trials of linezolid for RR-TB.

	Nitroimidazole	Bedaquiline	Clofazimine	Linezolid	Fluoroquinolone	Pyrazinamide	Other drugs
Nix-TB (completed) **NCT02333799**	PMD			1200			
ZeNiX **NCT03086486**	PMD			1200 vs 600			
endTB **NCT02754765**	DLM			600 then 300	MFX LFX		
endTB-Q **NCT03896685**	DLM			600 then 300			
TB-PRACTECAL **NCT02589782**	PMD			600 then 300	MFX		
BEAT-tuberculosis **NCT04062201**	DLM			600	LFX		
NExT (completed) **NCT02454205**				600	LFX		hdINH ETO
STREAM-2 **NCT02409290**					LFX		hdINH, ETO, EMB
SimpliciTB **NCT03338621**	PMD				MFX		

Table 2. BDQ = bedaquiline, DLM = delamanid, ethambutol, LFX = levofloxacin, LZD = linezolid, MFX = moxifloxacin, PMD = pretomanid, ETO = ethionamide, hdINH = high dose isoniazid

Although linezolid is a repurposed drug with substantial treatment experience in Gram-positive infection, there are major uncertainties around off-label use in TB treatment. These include safety of longer duration of therapy, sources of pharmacokinetic (PK) variability in TB patients, dosing that balances toxicity and efficacy in TB, and understanding resistance mechanisms. This thesis aims to delineate and address the key knowledge gaps that impede optimal use of this important antituberculosis agent. The following section provides an updated overview of linezolid use in RR-TB, complementing the detailed literature review in **Chapter 2**, providing context and rationale for the series of studies that follow.

Linezolid improves outcomes in RR-TB

Linezolid, developed in 1996, is the prototype member of the novel oxazolidinone antibiotic class. It has activity against a broad spectrum of Gram-positive bacteria and is approved for the treatment of serious infections caused by these pathogens at a dose of 600 mg twice daily [65, 66]. Linezolid also shows excellent activity against susceptible and resistant strains of *M. tuberculosis*, with minimum inhibitory concentrations (MICs) ≤ 1 μg/ml [67], first reported from *in vitro* and murine models at the end of the last century [68-71]. Through its unique inhibition of the initiation phases of protein synthesis, linezolid is bacteriostatic against *M. tuberculosis* in mouse models [70, 72] and had modest EBA in a small clinical study [73]. Recently, however, a phase 2a dose-finding trial (n = 114) demonstrated unequivocal bactericidal activity over 14 days at doses ranging from 300 mg daily to 1200 mg daily, with a clear exposure-response relationship for antimycobacterial activity [74].

As described in **Chapter 2**, several observational studies and two RCTs have shown that the inclusion of linezolid in treatment regimens for RR-TB results resulted in markedly improved rates of sputum culture conversion and favourable clinical outcomes. WHO commissioned an updated individual patient data meta-analysis of observational and experimental studies published between 2009 and 2016 to inform use of individual drugs for RR-TB; 50 studies were included, providing data from 12,030 patients. In this analysis, which included over 800 patients exposed to linezolid therapy, linezolid use was associated with significantly greater treatment success (crude odds ratio 1.5; 95% confidence interval (CI), 1.2 - 1.9) and lower mortality (crude odds ratio 0.4; 95% CI, 0.3 - 0.5) [38]. The endTB multicountry observational cohort study recently

reported early outcomes for RR-TB patients treated with the new drugs bedaquiline or delamanid in programmatic settings. Among 1,109 patients with positive baseline *M. tuberculosis* culture, 82% of whom also received linezolid in their regimen, 939 (85%) experienced culture conversion within 6 months of treatment initiation [75]. As mentioned above, the single arm Nix-TB trial combined linezolid 1200 mg daily with bedaquiline and pretomanid (a nitroimidazole) in an all-oral 6-month regimen for 109 patients with fluoroquinolone-resistant TB and complicated RR-TB, with 90% (95% CI, 83 to 95) favourable outcome at 6 months of post-treatment follow-up. This response is comparable to contemporary programmatic experience with longer bedaquiline-containing regimens [60, 76] and with first-line therapy for drug-susceptible TB.

These observations confirm an important role for linezolid in the treatment of RR-TB and are the basis for its prominence in treatment guidelines and inclusion in trials of novel drug regimens for RR-TB. However, there are several important limitations and uncertainties that need to be addressed to inform expanding use in TB programmes.

Toxicity with linezolid use in RR-TB

Linezolid interferes with mitochondrial protein synthesis [77] by binding to conserved regions of mammalian mitochondrial RNA shared with its bacterial ribosomal targets [78], thus disturbing energy production in tissues highly dependent on oxidative phosphorylation. This may result in clinical adverse events such as myelosuppression, optic and peripheral neuropathy, and lactic acidosis, which occur more commonly with linezolid use beyond the approved 28 days [79]. The duration of linezolid therapy in RR-TB usually exceeds 6 months (and may be continued for up to 24 months); this prolonged administration may lead to adverse events requiring treatment discontinuation and is a major concern to prescribers. In an analysis of provider consultations for RR-TB management in the United States, 4% of queries related to linezolid use, over 80% of which were for adverse events or dose reductions [80].

Despite widespread use and prescriber concerns, clinical toxicity has not been well characterised, particularly in programmatic settings where national registries are not designed to capture detailed patient-level data. The most recent systematic review was published in 2015 and included data from only 367 patients; the included cohorts were small, with only one study involving more than 50 patients [81]. Nearly half of patients

taking linezolid for RR-TB experienced adverse events; peripheral neuropathy and anaemia requiring discontinuation of therapy were reported in 31% and 27%, respectively. However, there was substantial heterogeneity in study population, definition of events, and linezolid dosing. Prevalence of adverse events were similar in a small Cape Town cohort where 60% of patients were HIV-positive, but severity and ascertainment were not reported. Linezolid toxicity was a major issue in the Nix-TB trial, which used a higher dose of 1200 mg daily. Among the 109 South African participants with complicated RR-TB, 50% of whom were HIV-positive, 25% permanently discontinued linezolid because of adverse events. Overall, grade 3 or higher adverse events occurred in 57%, mostly related to linezolid use; 88 (81%) participants reported symptoms of peripheral neuropathy and 40 (37%) developed anaemia of any severity. These findings may not be generalisable to programmatic settings where the 600 mg dose is used, linezolid is typically provided for longer durations, and there is less rigorous safety monitoring. However, the Nix-TB study raises important questions about linezolid use and optimal dosing in RR-TB therapy, and high-quality data on adverse events is needed for the standard 600 mg dose from programmatic settings.

Risk factors for linezolid toxicity

Mitochondrial toxicity is associated with reductions in the activity of mitochondrial complexes I, III, IV (cytochrome *c* oxidase), and V in experimental animals given linezolid [77]; key subunits of these complexes are encoded by mitochondrial DNA (mtDNA), raising the possibility that certain mtDNA polymorphisms may increase the risk of linezolid toxicity. Specific polymorphisms in 16S rRNA (A2706G and G3010A) have been described in case reports of patients with linezolid-induced lactic acidosis [82], but these are common in the general population and were not associated with adverse events in a cohort of 38 Korean patients given linezolid for XDR-TB [83]. However, the 2706A>G transition lies very close to the peptidyl transferase centre, a key functional site of mitochondrial 16S rRNA, implying a potential relationship between this polymorphism and linezolid inhibition of protein synthesis [78]. This has not yet been explored in African populations, which may have different population mtDNA polymorphisms affecting predisposition to linezolid toxicity.

Other risk factors for development of linezolid-associated adverse events are not well described, particularly for patients with TB. HIV positive patients are possibly at

increased risk because of underlying bone marrow dysfunction and peripheral neuropathy; use of nucleoside reverse transcriptase inhibitors may contribute to mitochondrial dysfunction [84]. In patients with Gram-positive infection older age, renal impairment, and baseline platelet counts have been associated with higher risk [85-89].

Linezolid pharmacokinetics and exposure-toxicity relationships in TB

Homology between the human mitochondrial binding site and the efficacy target in *M. tuberculosis* results in a narrow therapeutic window - therapeutic exposures close to toxicity thresholds - which is influenced by PK variability [83, 90, 91]. There is a linear relationship between linezolid exposure and dose (although some studies have suggested non-linearity, possibly due to autoinhibition of metabolism because of mitochondrial toxicity [92, 93]), but large inter-individual PK variability has been observed [94, 95]. The PK profile of linezolid in TB patients is poorly characterised, particularly in HIV co-infection which could influence PK and pharmacodynamics (PD) of TB drugs [96-98]. In a recent systematic review of linezolid PK in TB therapy, only 2 studies (n = 48) evaluated the standard dose of 600 mg daily; all patients were HIV-negative, and data from a full PK profile were only available from 10 patients [99]. Additional PK and clinical data are also required to identify sources of PK variability in TB patients, needed to optimise linezolid dosing in relevant populations. For example, linezolid clearance is strongly influenced by body weight, age, severity of illness, renal function, and albumin concentrations in patients with Gram-positive infection, but this is not adequately studied in TB patients [100-104].

To determine the dose of linezolid, PK targets for efficacy and safety need to be established and the relationship between linezolid exposure and these targets defined in TB patients. Efficacy in TB appears to be driven by area under the concentration-time curve (AUC) [105, 106]; a free AUC/MIC target of 119 was derived from a hollow-fibre infection model that relates drug concentrations to mycobacterial killing, but not on clinical outcomes [107]. Establishing PK targets for clinical efficacy is challenging in the context of multi-drug regimens and outcomes in TB that are influenced by multiple non-drug factors.

Because adverse events associated with linezolid (haematological and neurological) do not overlap with other drugs used in RR-TB, it is less complex to delineate relationships

between drug exposure and toxicity (compared with efficacy). Trough concentrations have shown an inverse correlation with mitochondrial function [83] and clinical toxicity among patients with Gram-positive infection [108, 109]. A trough target threshold of 2 mg/L for toxicity has been suggested based on the high proportion of clinical events observed above that concentration among Korean XDR-TB patients in a small RCT (n = 38) [63, 83, 110]. Although this threshold is now widely used in PK/PD analyses, the specificity is poor, and the target has not been validated in other cohorts. Linezolid PK-toxicity targets have therefore not been adequately established for TB and have never been studied in populations from sub-Saharan Africa with high rates of HIV co-infection, a factor which could influence linezolid PK variability and toxicodynamic effects.

The ability of the standard linezolid dose to attain provisional PK-efficacy and toxicity targets for TB is also uncertain. WHO recommendations for the 600 mg daily dose are based on a systematic review that included only 2 studies (n = 48) reporting PK data at that dose, with large heterogeneity in $AUC_{0\text{-}24}$ and C_{min} (trough) estimates, and without data from African patients [95, 99].

Linezolid resistance in RR-TB

There are concerns that widespread use of linezolid in RR-TB programmes, particularly with use of lower doses (necessitated by toxicity issues), may select out resistant strains. The risk and frequency of linezolid resistance in TB treatment is unknown. An updated systematic review of studies reporting linezolid resistance in TB was performed for this thesis (not including the study presented in Chapter 5). Search terms included ‘mutation’, ‘rrl’, ‘rplC’, ‘L3 protein’, ‘23s rRNA’, ‘resistant’, ‘mycobacterium tuberculosis’, and ‘linezolid’; studies reporting genotyping for linezolid resistance in clinical isolates were included. As summarised in Table 3, linezolid resistance has only been described in 33 patients with TB (16 patients prior to 2019, when our study was performed). Only three published reports provided clinical data for patients with linezolid resistance, including data from only 27 patients [63, 111, 112].

Table 3. Studies reporting linezolid resistance in clinical isolates.

Mutation	MIC range (mg/L)	*In vitro* studies	Clinical studies	Unique patients	Resistance-conferring	References
rrl						

G2814T (2576)	4 – 16	2	4	6	Definite	[63, 113-117]
A2810C/T (2572)	4 – 16	2	2	2	Definite	[115, 116, 118, 119]
G2299T (2061)	16 – 32	2	2	NR	Definite	[113, 117, 120, 121]
G2270C/T (2032)	4	2	0	NA	Probable	[118, 119]
A2689T (2451)	NR	1	0	NA	Probable	[113]
G2685T (2447)	16	0	1	1	Definite	[63]
G2746A (2508)	1	1	0	NA	Possible	[119]
C2848A (2610)	1	1	0	NA	Possible	[119]
C1921T (1683)	NR	0	1	1	Unlikely	[114]
G2294A (2056)	NR	0	1	1	Possible	[114]
rplC						
T460C [Cys154Arg]	2 - 32	6	7	22	Definite	[63, 112-114, 118-126]
C463G [His155Asp]	2	2	0	NR	Possible	[120, 121]
rplD						
G377A [Arg126His]	> 16	1	0	NR	Probable	[120]

Table 3. *E. coli* numbering in parentheses; amino acid substitutions in square brackets. NR, not reported; NA, not applicable. Definitions of resistance conferring:

Definite: MIC > 1-dilution above the critical concentration (CC) in more than 1 study or MIC > 1 mg/L plus evidence clinical failure

Probable: MIC > 1-dilution above CC or MIC > 1 mg/L without evidence of clinical failure

Possible: MIC 1 - 2 mg/L and near peptidyl transferase centre (PTC) without evidence of clinical failure or unknown MIC and near PTC with evidence of clinical failure

Unlikely: MIC ≤ 1 or MIC 1 - 2 mg/L and distant from PTC

Genotypic correlates of linezolid resistance, needed for development of rapid molecular resistance testing, have also not been systematically studied. Figure 1 shows known resistance-associated mutations in 23S rRNA (encoded by *rrl*), identified in the systematic review. From limited published data, most isolates with phenotypic resistance are associated with a limited number of mutations in two *M. tuberculosis* genes, *rrl* and *rplC*, but a substantial minority had no detectable mutations (Table 3). It is necessary to characterise the mutations associated with linezolid resistance in isolates from patients with clinical treatment failure and determine the risk factors for linezolid resistance to support use in RR-TB programmes.

Figure 1. Mutations associated with linezolid resistance in 23S rRNA, encoded by *rrl*.

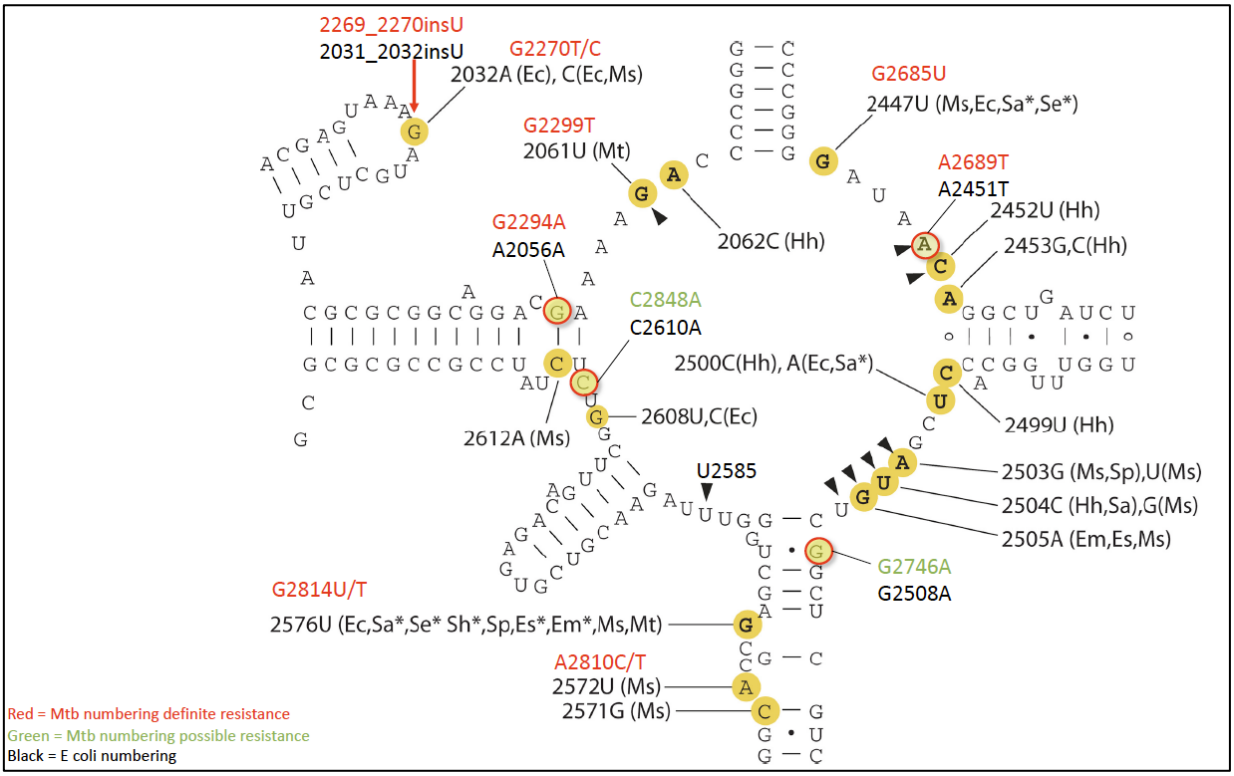


Figure 1. Secondary structure of the peptidyl transferase loop of domain V of 23S rRNA (*M. smegmatis* sequence in *E. coli* numbering in black text). Nucleotides that form the linezolid binding pocket are indicated with black triangles. Nucleotide positions where mutations confer linezolid resistance in any organism are marked with yellow circles. Sequence numbers in green or red text indicate mutations associated with *M. tuberculosis* (in *M. tuberculosis* numbering); red text indicates definite resistance; green indicates possible resistance (defined in Table 3 legend above). Figure adapted from Katherine S. Long and Birte Vester *Antimicrob. Agents Chemother.* 2012, 56(2):603.

Research priorities for linezolid in RR-TB

Recognising the above challenges, WHO has called for "additional research on the optimal dose and duration of linezolid for use in DR-TB regimens" and listed the following research priorities in its latest guidelines [62]:

- PK and safety studies to determine optimal drug dosing.
- Rigorous reporting and patient predictors of adverse events.
- Improved diagnostics and drug susceptibility testing for drugs without currently available rapid molecular methods.

Objectives and specific aims of the PhD project

The overarching objective of this PhD was to characterise the PK, frequency and predictors of adverse events, and correlates of linezolid resistance among South African patients with RR-TB and high rates of HIV co-infection to inform the expanded use of linezolid in TB programmes. To address this, a series of studies were undertaken with the following specific aims:

1. Describe the PK of linezolid, explore the effect of key covariates on PK parameters, and estimate the probability of PK/PD target attainment corrected for the *M. tuberculosis* MIC distribution **(Chapter 3)**.
2. Determine the frequency and timing of linezolid-associated adverse events in a programmatic setting with high prevalence of HIV **(Chapter 4)**.
3. Explore the effect of linezolid exposure, clinical characteristics, and risk-associated mtDNA single nucleotide polymorphisms (SNPs) (A2706G and G3010A in 16S rRNA) on adverse events, including peripheral and optic neuropathy, bone marrow suppression, and hyperlactataemia **(Chapter 4)**.
4. Identify mutations associated with phenotypic linezolid resistance in *M. tuberculosis* isolates from patients with treatment failure on a linezolid-based RR-TB regimen **(Chapter 5)**.
5. Describe the clinical characteristics of patients with RR-TB treatment failure and linezolid resistance and explore the risk factors for the development of linezolid resistance **(Chapter 5)**.

Overview of study designs and settings

The PhD studies relating to Aims 1 - 3 were nested within two parent RR-TB cohorts; a retrospective study was done to address Aims 4 - 5. These are summarised below and described in detail in the relevant chapters.

Design of parent studies

The New Treatment Regimen for Patients with Multi-drug Resistant Tuberculosis (NExT) trial (NCT02454205) was an open-label RCT evaluating a novel 6 to 9-month injection-free regimen containing linezolid (600 mg daily, reduced to 300 mg in the event of toxicity), bedaquiline, levofloxacin, pyrazinamide, and ethionamide (or high dose isoniazid) compared with a conventional empiric injection-based regimen for RR-TB. The trial recruited adults with newly diagnosed RR-TB from five South African treatment facilities and associated decentralised primary care clinics, with enrolment commencing in 2015. Participants were followed for 24 months for the primary outcome of treatment success. The planned sample size was 300 participants, but the trial was discontinued prematurely in December 2020 after 154 participants were enrolled because of slow recruitment rate [127]. Consecutive participants randomised to the intervention arm at the Brooklyn Chest Hospital site in Cape Town were recruited between 2016 and 2017 for the cross-sectional linezolid PK study presented in **Chapter 3**.

PROBeX was an observational cohort study among adult patients receiving bedaquiline-based regimens for RR-TB, most of whom had fluoroquinolone resistance. The primary objectives were to determine the drug-drug interactions between bedaquiline and the antiretroviral protease inhibitor lopinavir/ritonavir and the effect of this on QT prolongation, as well as identifying genetic mechanisms of bedaquiline resistance [128]. The study enrolled 195 participants from three public sector TB referral hospitals in South Africa between 2016 - 2018, who were followed for up to 24 months. A subgroup of consenting PROBeX participants at the Brooklyn Chest Hospital site who were also receiving linezolid as part of their treatment were enrolled in the cross-sectional linezolid PK study (**Chapter 3**); participants on linezolid-based therapy from all sites were included in the longitudinal linezolid toxicity analysis (**Chapter 4**).

Study population

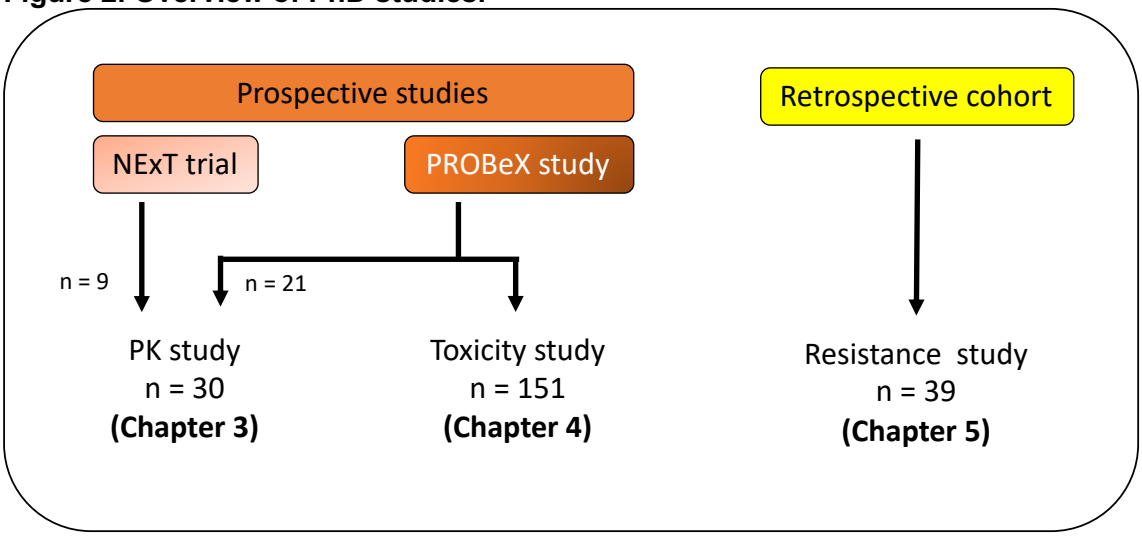
Both prospective cohort studies included patients ≥ 18 years of age with known HIV status and newly diagnosed pulmonary RR-TB. The NExT trial excluded pregnant and

breastfeeding women, patients with a history of previous RR-TB treatment, and a Karnofsky performance score ≤ 50. Potential participants were identified by local clinicians and referred to the respective study teams for screening and enrolment. Separate consent was obtained for intensive PK sampling; the protocol and consent procedures for longitudinal components of the linezolid analyses were fully aligned with the parent PROBeX study.

For the linezolid resistance study (**Chapter 5**), patients over the age of 13 years with treatment failure on a linezolid-containing regimen were retrospectively identified from two public sector TB clinics until 2018: Brooklyn Chest Hospital in Cape Town and Jose Pearson Hospital in Port Elizabeth. Cases with reported treatment failure or phenotypic linezolid resistance were also identified from the National Tuberculosis Reference Laboratory at the National Institute for Communicable Diseases (NICD) from a national surveillance programme for second-line drug resistance. Case records and *M. tuberculosis* isolates were retrieved from eligible patients for analysis.

All participants in PROBeX and the retrospective cohort were treated according to National TB Programme guidelines, which, during the recruitment period, were aligned to the conventional longer regimen recommended by WHO. Bedaquiline and linezolid (at 600 mg daily, reduced to 300 mg daily at clinician discretion) were offered to patients with fluoroquinolone-resistant TB (at that time termed pre-XDR or XDR-TB) or those with RR-TB and poor clinical response or drug intolerance.

Figure 2. Overview of PhD studies.



Study procedures

PK study (Chapter 3)

Consecutive participants enrolled in the intervention arm of the NExT trial and those on linezolid-based therapy in the PROBeX study were approached for participation in the linezolid PK study at Brooklyn Chest Hospital in Cape Town. After providing informed consent, participants underwent intensive venous sampling to characterise the steady state PK of linezolid over a 24-hour dosing interval on a single occasion around Month 2 of linezolid therapy. Most participants in PROBeX were inpatients at the time of the intensive sampling visit and all NExT participants attended as outpatients. At the first visit, participants had a peripheral intravenous catheter inserted from which approximately 4 mL of blood was drawn prior to dosing, then at 1, 2, 3, 4, 5, 6, and 24 hours after the observed dose (some participants in the PROBeX study had additional sampling at 8 and 48 hours) (Figure 3). Participants returned for a second visit the following morning to collect a single sample at 24 hours after the previous observed dose and before the next dose was taken. This was collected by single venepuncture. The blood samples were drawn into EDTA tubes, centrifuged within 30 minutes of sampling, and transported on dry ice or in a portable -80˚C freezer for storage until analysis. We obtained basic biometric data including sex, race, weight, and height, and recorded the exact timing of the previous dose, whether the pills were crushed, and the nature of meals taken around dosing. The use of other concomitant drugs was also documented.

Figure 3. Intensive PK sampling schedule

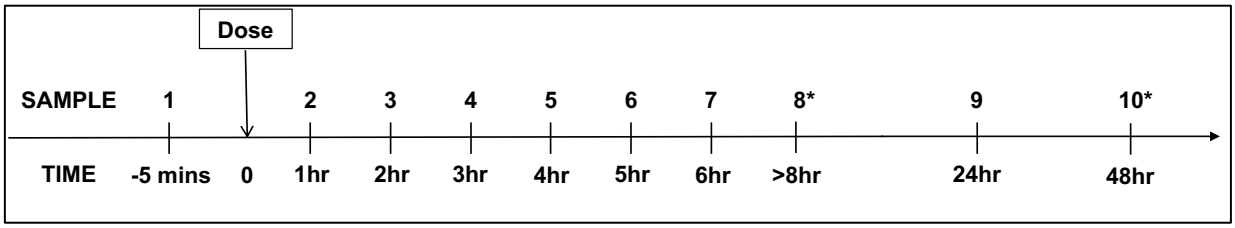


Figure 3. *Additional timepoints in the PROBeX study. hr = hour; mins = minutes.

Toxicity study (Chapter 4)

PROBeX participants completed up to 9 visits after enrolment: monthly through the first 6 months of treatment then every 6 months for up to 24 months of follow up (Figure 4). The following information was captured at the baseline visit: sociodemographic information, medical co-morbidities, HIV status, TB history and details of index TB

episode, biometric data (weight, height), ethnicity, and details of concomitant drug therapy. Baseline and monthly screening for peripheral and optic neuropathy was conducted. The Brief Peripheral Neuropathy Screen (BPNS), a brief self-report tool that has been validated for the identification of sensory peripheral neuropathy in HIV [129], was used to screen for peripheral neuropathy. Bedside tests were done to assess visual acuity (using logMAR charts) and colour vision (using 14-plate Ishihara charts) to screen for optic neuropathy. Treatment adherence, regimen changes, and updated clinical information were documented at each follow up visit.

Figure 4. Study schema for linezolid procedures in the PROBeX study.

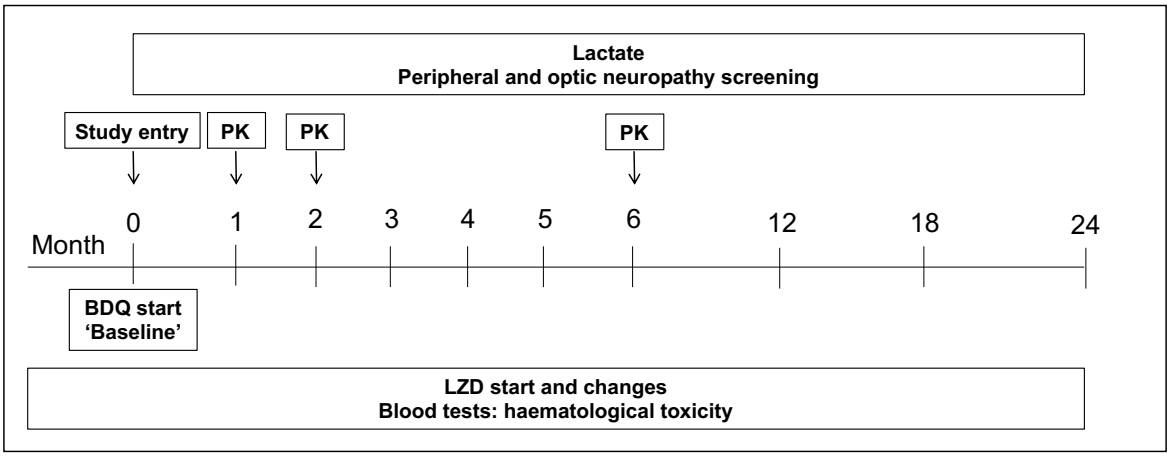


Figure 3. LZD = linezolid; BDQ = bedaquiline; PK = pharmacometric

Phlebotomy was performed at study entry and monthly visits for full blood count and lactate quantification; these tests, as well as baseline CD4 count and HIV viral load, were performed by the National Health Laboratory Service (NHLS). Sparse PK sampling was conducted on three occasions at Months 1, 2, and 6; a single pre-dose sample was drawn at each of these visits, centrifuged on site, stored at -80˚C, and transported to the Division of Clinical Pharmacology at UCT for determination of linezolid concentrations. Whole blood was collected at study entry (from participants who provided explicit consent) and stored for mitochondrial DNA extraction and sequencing of the 16S rRNA gene; these assays were performed by the Centre for Proteomic and Genomic Research in Cape Town.

Linezolid drug susceptibility testing (Chapters 3 and 5)

Linezolid MIC values were determined on available *M. tuberculosis* isolates obtained at study entry for probability of target attainment analysis in the PK study (**Chapter 3**).

These assays were performed at the South African Medical Research Council Centre for Tuberculosis Research at Stellenbosch University using the BACTEC MGIT 960 system (BD Diagnostic Systems, Sparks, MD, USA) and continuous growth monitoring with Epicenter software [130].

For the retrospective linezolid resistance study (**Chapter 5**), isolates retrieved from NHLS laboratories and the NICD were shipped in original liquid culture bottles to the BSL3 laboratory at the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town for linezolid resistance testing. Phenotypic linezolid resistance was determined on sub-cultured isolates by MIC assessment using a microtitre assay [131]; DNA extraction and targeted Sanger sequencing was performed for genotypic resistance testing. Detailed descriptions of these methods are provided in the relevant chapters.

Data collection and data curation

Clinical information was obtained directly from study participants and from medical records. Clinical and routine laboratory data was captured on bespoke case report forms designed in REDCap. On completion of each study, the data were exported to Excel spreadsheets checked for accuracy and completeness using Stata software. Other data, including drug concentrations, MIC and genotypic resistance testing, and mtDNA polymorphisms, were imported directly into Stata and appended to relevant datasets.

Overview of statistical analyses

Detailed analytical approaches are described for each study in respective chapters. Data were analysed using Stata 14 and 17 (StataCorp) to address the main research questions:

What are the sources of variability in linezolid PK among patients with RR-TB and a high prevalence of HIV, and what is the probability of PK/PD target attainment accounting for the measured MIC distribution?

Intensive PK sampling was performed on a subgroup of participants in two cohort studies of RR-TB. Non-compartmental analysis was done to describe PK parameters from observed drug concentration data. Associations between clinically relevant

covariates and linezolid exposure were explored using linear regression. The proportion of participants with estimated PK exposures above defined efficacy and toxicity targets was computed and probability distributions plotted **(Chapter 3)**.

What is the frequency, timing, and risk of linezolid-associated adverse events in a programmatic setting with high prevalence of HIV?

Longitudinal data analysis was conducted on a prospective cohort of patients treated with linezolid-containing regimens for RR-TB. Survival methods were used to describe and plot incidence of non-repeating events. Linear and logistic mixed-effects models were fitted to longitudinal data to quantify changes over time and explore individual risk factors for linezolid toxicity **(Chapter 4)**.

What is the frequency and risk factors for linezolid resistance among patients with treatment failure for RR-TB, and what are the genotypic correlates of linezolid resistance?

Patients with RR-TB treatment failure on linezolid-based therapy were identified from medical and laboratory records, and *M. tuberculosis* isolates obtained. Bivariate analysis was done to compare clinical and treatment parameters between patients who developed linezolid resistance with those who did not. MIC distributions were plotted, stratified by presence of genotypic resistance mutations **(Chapter 5)**.

Ethical approval

All studies were approved by the University of Cape Town Human Research Ethics Committee (UCT HREC), reference numbers 805/2016; 264/2015; 920/2015. Participants provided written informed consent prior to performance of any study procedure.

Summary of thesis structure

Chapter 2 is a systematic narrative review of linezolid use for RR-TB, providing detailed background on efficacy, safety, PK, and resistance. The review aimed to identify important research gaps as a scoping exercise to provide rationale for the studies in this PhD thesis.

Chapter 3 presents findings of the non-compartmental analysis and probability of target attainment from the linezolid PK study. The study is published but presented in a format

consistent with the thesis document. Supplementary figures from the publication are included.

Chapter 4 is a longitudinal analysis of linezolid toxicity from the main prospective cohort study. These findings are not yet published and are presented as a chapter, including data intended as supplementary material in the published version.

Chapter 5 presents findings from the retrospective linezolid resistance study. The published study is presented in a format consistent with the thesis document, including supplementary material.

Chapter 6 presents a summary of all the preceding chapters as conclusions to the thesis.

CHAPTER 2

Linezolid in the treatment of drug-resistant tuberculosis: the challenge of its narrow therapeutic index

ABSTRACT

Introduction: Linezolid is an oxazolidinone with potent activity against *M tuberculosis* and improves culture conversion and cure rates when added to treatment regimens for drug resistant tuberculosis. However, linezolid has a narrow therapeutic window, and the optimal dosing strategy that minimises the substantial toxicity associated with linezolid's prolonged use in tuberculosis treatment has not been determined, limiting the potential impact of this anti-mycobacterial agent.

Areas covered: This paper aims to review and summarise the current knowledge on linezolid for the treatment of drug-resistant tuberculosis. The focus is on the pharmacokinetic-pharmacodynamic determinants of linezolid's efficacy and toxicity in tuberculosis, and how this relates to defining an optimal dose. Mechanisms of linezolid toxicity and resistance, and the potential role of therapeutic drug monitoring are also covered.

Expert commentary: WHO has recently upgraded the status of linezolid to a core second line agent, and it is likely to become a key component of treatment regimens for drug resistant tuberculosis in sub-Saharan Africa and other high burden regions. Prospective pharmacokinetic-pharmacodynamic studies are required to define optimal therapeutic targets and to inform improved dosing strategies that minimise the risk of toxicity and the emergence of linezolid resistance.

Introduction

The pandemic of drug resistant tuberculosis is a major barrier to global tuberculosis control. While the number of drug-susceptible tuberculosis cases appears to be decreasing, the number with multidrug resistant (MDR) tuberculosis (resistance to both rifampicin and isoniazid) has remained virtually unchanged in recent years, and globally an estimated 3.3% of new tuberculosis cases and 20% of previously treated cases had MDR tuberculosis in 2014 [26]. This equates to almost half a million incident MDR tuberculosis cases with approximately 190,000 deaths, a case fatality ratio over 2.5-fold higher than for drug-susceptible tuberculosis [26]. The worst outcomes are seen with extensively drug resistant (XDR) tuberculosis (additional resistance to fluoroquinolones plus second line injectable agents), the prevalence of which is estimated to be 10% of MDR tuberculosis cases globally [26], which has an overall 5-year mortality of 70% [30].

Under programmatic conditions only 50% of MDR tuberculosis cases have treatment success [26], driven by poor efficacy of second line tuberculosis drugs, prolonged duration of therapy with high rates of loss to follow up, and high rates of toxicity [26]. WHO has recently strengthened its standard 18-24 month MDR tuberculosis regimen with additional drug options [44] and has introduced a new short-course regimen of 9-12 months for non-pregnant patients with pulmonary tuberculosis susceptible to aminoglycosides and fluoroquinolones [44], but the efficacy of this shortened regimen is not informed by randomized controlled trials. Overall, 30% of patients with MDR tuberculosis require removal of drugs from their regimen (particularly the aminoglycosides, which remain in the updated WHO MDR tuberculosis guidelines) because of adverse events [132] with no other effective treatment options until recently. The overlapping toxicity of anti-tuberculosis drugs used for drug-resistant tuberculosis and antiretroviral therapy (ART) further limits the use of current regimens, particularly in Southern Africa where the tuberculosis epidemic is driven largely by HIV and co-infection rates exceed 60% [26]. In addition to being more toxic, less effective and much more expensive [15, 133] than the treatment for drug-susceptible tuberculosis, the use of standard drug-resistant tuberculosis treatment regimens results in resistance amplification during therapy [31, 32], thereby worsening the problem of drug-resistant tuberculosis.

There is thus a critical need for more effective, safe, and well-tolerated drugs in order to support shorter and injection-free treatment regimens, improve adherence, and interrupt the cycle of resistance amplification and transmission of drug-resistant tuberculosis. Linezolid, an oxazolidinone antibiotic initially developed to treat Gram-positive bacterial infections, improves culture conversion and cure rates in complicated MDR as well as XDR tuberculosis [81], and has the potential to support injection-free regimens. Because of its anti-mycobacterial potency and impressive impact on outcomes in small clinical trials, linezolid is likely to become a key component of treatment regimens for drug-resistant tuberculosis. However, linezolid is not licensed as an anti-tuberculosis agent and its expanded use in tuberculosis therapy is limited by the substantial toxicity that occurs beyond the approved 28-day duration for Gram-positive infections. It is also unclear what dose of linezolid optimizes the delicate balance between adverse events, efficacy, and the suppression of resistance. The latest WHO MDR tuberculosis guidelines have included linezolid in the list of core second line agents (without dosing recommendations), but caution against its use where there are other available options or close toxicity monitoring is not possible.

In this review, we aim to present the current evidence for the use of linezolid in the treatment of tuberculosis in adults. Our focus is on the anti-mycobacterial activity and pharmacokinetics (PK) of linezolid in tuberculosis treatment, and how this relates to efficacy and toxicity. We also explore the mechanisms of linezolid toxicity and resistance and discuss dosing strategies and other approaches to potentially reduce these complications and optimize the use of linezolid in tuberculosis therapy.

Search strategy

We performed a broad search in Pubmed combining the terms ‘linezolid’ and ‘oxazolidinone’ with ‘tuberculosis,’ ‘pharmacokinetics,’ ‘resistance,’ ‘toxicity,’ ‘mitochondrion,’ and ‘therapeutic drug monitoring.’ Search limits were set to only include studies published in English. We also hand searched bibliographies of all included articles for additional relevant publications.

Mechanism of action

Linezolid is the prototype member of the synthetic oxazolidinone antibiotic class. Like all oxazolidinones, linezolid inhibits initiation of bacterial protein synthesis through a

unique mechanism of action by binding to the 23S RNA peptidyl transferase centre (PTC) of the 50S subunit of the prokaryotic ribosome [134, 135]; it has also been shown to have downstream effects by binding the P (peptidyl) site on mature 70S initiation complexes [136]. These actions ultimately prevent translation of mRNA leading to bacteriostatic effects [137]. Although oxazolidinones interact with the peptidyl transferase inhibitors clindamycin and chloramphenicol by competing for overlapping binding sites on the 50S ribosomal subunit [138], their mechanism of action is different and there is no cross resistance between these antibiotics [139], or with any anti-tuberculosis drugs.

Pharmacokinetic (PK) profile and drug-drug interactions

The general pharmacokinetics of linezolid have been thoroughly reviewed [140-142]. Peak serum concentrations of 10.3 – 14.7 mg/L are reached after ~2 hours following oral administration of 600 mg 12-hourly, with an elimination half-life of 4.8 – 5.5 hours. Linezolid has good tissue penetration [143, 144], including into the cerebrospinal fluid [145]. Several PK characteristics of linezolid are favourable for treating pulmonary tuberculosis: There is almost complete oral bioavailability with good pulmonary penetration [146-148], the median sputum-to-serum linezolid concentration ratio is 1.0 (range, 0.7 to 1.5) [149], and oral dosing achieves linezolid concentrations above the wild type minimum inhibitory concentration (MIC) of *M tuberculosis* in epithelial lining fluid [148, 150], and within alveolar macrophages [143, 147].

About 65% of an administered dose undergoes non-enzymatic oxidative metabolism [142] resulting in the formation of two inactive metabolites. This process contributes to the substantial inter-individual variability observed with this lipophilic drug [100, 140]. Urinary excretion is the major elimination route for non-metabolized drug, with ~30% of the dose appearing unchanged in the urine, and ~50% excreted as inactive metabolites [151]. Dose adjustment is not recommended in renal impairment [140, 142] but a number of studies have found reduced creatinine clearance to be a risk factor for haematological adverse events [85-88]. The association between renal impairment and linezolid toxicity requires further investigation, but may have implications for its use in sub-Saharan Africa, where renal impairment commonly accompanies HIV-associated tuberculosis due to HIV-associated nephropathy [152].

The cytochrome P450 isoenzyme CYP3A4 has a minor role in linezolid metabolism [153]. It has been suggested that linezolid is a substrate of the membrane efflux transporter P-glycoprotein [154, 155], but this has not been confirmed. Co-administration with clarithromycin, which inhibits P-glycoprotein and CYP3A4, results in increased linezolid exposure [156], with up to a 50% increase in linezolid concentrations [154]. Ritonavir, an HIV protease inhibitor used as a pharmacological booster in ART, which is also an inhibitor of P-glycoprotein and CYP3A4, will likely also increase linezolid exposure. Linezolid exposures are modestly reduced when co-administered with rifampicin (32% reduction in linezolid AUC and 21% reduction in C_{max}), a potent inducer of P-glycoprotein and CYP3A4 [153, 155]; this effect may persist for up to 3 weeks after discontinuation of rifampicin [157].

Linezolid is a reversible non-selective inhibitor of monoamine oxidase (MOA), and cases of serotonin toxicity have been uncommonly reported during co-administration with selective serotonin reuptake inhibitors and other agents that increase serotonin concentrations in the central nervous system [79, 158-161]. MOA inhibition by linezolid may also lead to potentiation of vasopressor effects of sympathomimetic and adrenergic agents [162]. A single study demonstrated an antagonistic effect between linezolid and levofloxacin in a macrophage model of *M tuberculosis* [163]. The clinical consequences of this are unknown, but reassuringly, 3-drug combinations involving these two agents were as effective against *M tuberculosis* isolates as first line therapy *in vitro* [164]. Finally, there is a theoretical risk of combined mitochondrial toxicity when linezolid is co-administered with other antimicrobials (such as aminoglycosides, chloramphenicol, and clindamycin) that impair mitochondrial function.

Activity against *M. tuberculosis*

Linezolid was developed for its activity against multidrug resistant staphylococci, streptococci, and enterococci, and was approved in 2000 for the treatment of serious infections caused by these pathogens at a recommended dose of 600 mg twice daily for up to 28 days [65, 66]. It also has potent *in vitro* activity against *M tuberculosis*. The MICs against clinical *M tuberculosis* isolates are consistently lower than for Gram-positive pathogens, ranging from ≤ 0.125 to 1 mg/L [67, 165, 166], and an epidemiological cut-off (ECOFF) value of 0.5 mg/L has been suggested [167]. Although

critical concentrations have not been firmly established [168], an MIC of 1 mg/L is widely considered to represent the clinical susceptibility breakpoint.

In keeping with its mechanism of action, linezolid is bacteriostatic against *M tuberculosis in vitro* and in mice at doses equivalent to 300 mg daily in humans and weakly bactericidal at higher doses [70, 72, 169]. In a clinical study involving 29 HIV-uninfected adults with smear-positive pulmonary tuberculosis, linezolid demonstrated modest early bactericidal activity (EBA) at both 600 mg daily and twice daily doses, with very little further effect after 5 days [73]. Linezolid's limited bactericidal activity only occurred during the exponential growth phase in one *in vitro* study [169], implying a lack of sterilizing ability. However, recent clinical evidence suggests that linezolid may contribute to tuberculosis cure, particularly in the context of novel drug regimens [170]. Linezolid has been shown to have potent bactericidal activity against non-replicating bacilli *in vitro* and in mice [171], and its addition to various tuberculosis regimens in a murine experiment led to significant reductions in lung quantitative cultures at 2 months, and resulted in lung sterilization in combination with second line anti-tuberculosis drugs [172]. Strikingly, in a recently published series of murine experiments, linezolid significantly enhanced the bactericidal and sterilizing ability of a 3-drug regimen that included the new anti-tuberculosis agents bedaquiline and pretomanid. Furthermore, this combination showed superior sterilizing activity compared to first line agents for drug-susceptible *M tuberculosis* strains [173].

Improved clinical outcomes with linezolid use in drug-resistant tuberculosis

Linezolid's potent anti-mycobacterial activity has translated into clinical efficacy. A number of observational studies and two small randomized controlled trials (RCTs) have shown that the inclusion of linezolid in treatment regimens for drug-resistant tuberculosis resulted in improved rates of sputum culture conversion and more favourable clinical outcomes [81]. There has been no systematic comparison of linezolid dosing regimens in tuberculosis treatment, and a range of doses have been used in these studies. Linezolid therapy is often continued until treatment completion, which in drug-resistant tuberculosis can take up to 24 months. Because of the recognition of increased linezolid toxicity with prolonged use of 600 mg twice daily (the approved dose for Gram-positive infections), the most widely used dosing strategy in tuberculosis is 600 mg daily with a reduction to 300 mg daily in the event of adverse events. At these

reduced doses linezolid exposures may exceed the MIC of *M tuberculosis*, and the addition of linezolid to the treatment of drug resistant tuberculosis has resulted in improved outcomes.

In a Korean RCT, which randomized 39 HIV-negative patients with intractable XDR tuberculosis to the delayed or immediate addition of linezolid, significantly more participants in the immediate-start group converted to a negative sputum culture by 4 months (79 vs 35%, P = 0.001). After 6 months of linezolid treatment, 87% of all patients had negative sputum cultures, including the subgroup who underwent a second randomization to a 300 mg daily dose. This effect was durable, with 71% remaining cured at 1 year after completion of treatment [63, 64]. These results are surprising, given that linezolid was added as a single drug to failing regimens, a strategy that usually fails in tuberculosis treatment. Similar impacts outcomes were seen in the second small RCT conducted in China (n = 65), where doses of 600 mg twice daily were used initially for up to 6 weeks, followed by a reduction to 600 mg or 300 mg daily depending on weight [174]. These results are consistent across studies and settings. The most recent meta-analysis reported that 83% of patients with drug-resistant tuberculosis (46% of whom had XDR tuberculosis) had successful outcomes (cured or completed) on linezolid-based therapy, and there was a pooled culture conversion rate of almost 90% [81]. At the time of this review, in the only reported data from sub-Saharan Africa, where there are high rates of HIV co-infection, the addition of linezolid (plus bedaquiline in some) to treatment regimens for patients with pre-XDR and XDR tuberculosis in a large township in Cape Town, South Africa, led to more rapid culture conversion (hazard ratio (HR) 3.33, 95% confidence interval (CI) 1.10–10.20) [175], higher culture conversion rates (64 versus 40%, p = 0.002), and a trend towards lower mortality (20 versus 31%) and treatment failure (11 versus 21%) at 9 months [176].

Taken together, these findings suggest an important role for linezolid in the treatment of drug-resistant tuberculosis and are the basis for its expanding use in clinical practice for complicated drug-resistant tuberculosis cases, and its inclusion in trials of novel drug regimens for MDR and XDR tuberculosis. However, published reports of linezolid use for drug resistant tuberculosis include outcomes data for only 367 patients worldwide; fewer than 10% of these were HIV-infected and only 55 cases have been reported from Africa [175, 176]. The two RCTs involved just 74 patients of Asian origin with a low

mean body weight, all of whom were HIV-uninfected. This may limit generalizability because linezolid exposure is inversely related to body weight [103, 177] and HIV infection is known to be a cause of PK variability in anti-tuberculosis drugs [98]. Additionally, these studies had designs where participants underwent dose reduction after an initial period on higher doses, and also lacked detailed PK analyses, making it difficult to compare the efficacy of different dosing strategies. Finding the lowest effective dose of linezolid is a priority in tuberculosis therapy and is necessitated by the occurrence of serious linezolid-associated adverse events.

The mitochondrial toxicity of linezolid

To minimize host toxicity, antimicrobials should be designed to selectively bind to microbial targets. The specificity of target binding is more difficult to achieve for antibiotics that interfere with bacterial protein synthesis as the molecular machinery for these processes have deep evolutionary roots that are a common feature of life. Bacterial protein synthesis inhibitors can cause toxicity in humans through binding to conserved homologous structures in mammalian mitochondrial RNA [78], which are largely encoded by mitochondrial DNA that originated from a eubacterial ancestor [178]. Inhibition of mitochondrial protein synthesis can lead to disruptions of the electron transfer chain and impairment of oxidative phosphorylation, resulting in tissue-specific dysfunction related to differential dependence on mitochondrial energy, or “threshold capacity” [179]. This manifests as clinical phenotypes similar to those seen in inherited mitochondriopathies, with myelosuppression, neuropathy, and hyperlactatemia predominating.

The selective activity of linezolid is compromised because of binding to conserved regions of mammalian mitochondrial ribosomes. The primary binding site for linezolid is the central loop of domain V of bacterial ribosomal 23S RNA (the PTC) [180], which has a high degree of homology with mammalian mitochondrial 16S rRNA. The areas of greatest similarity map to key functional domains, and the inhibition of bacterial protein synthesis by linezolid thus also results in inhibition of mitochondrial protein synthesis, particularly of key subunits of mitochondrial respiratory complexes which are translation products of mitochondrial ribosomal 16S RNA [78, 181]. This is depicted in Figure 1.

Figure 1. Shared binding sites of linezolid in *M tuberculosis* and human mitochondria and the downstream effects of inhibition of protein synthesis.

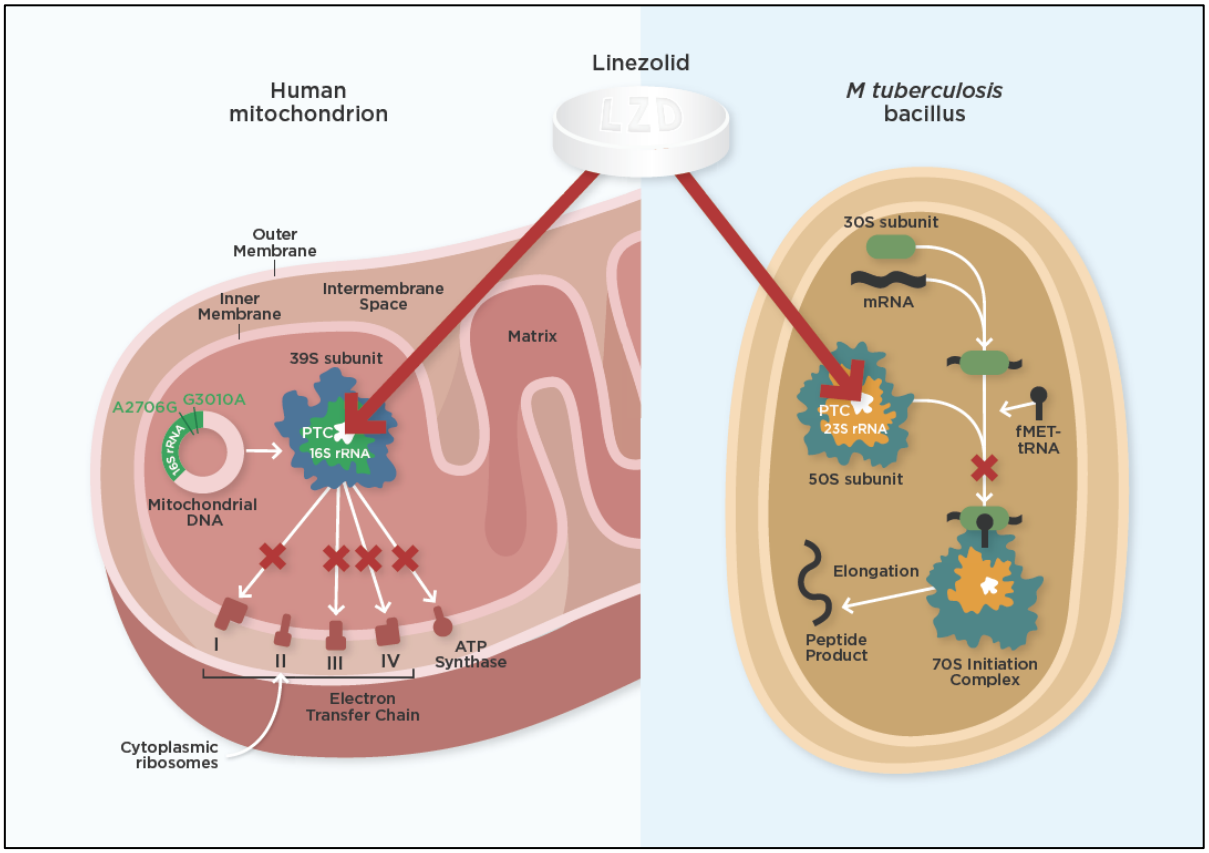


Figure 1. In *M tuberculosis*, linezolid prevents the formation of the 70S protein initiation complex by binding to the peptidyl transferase centre (PTC) in 23S RNA of the 50S ribosomal subunit, leading to its anti-mycobacterial activity. Linezolid toxicity occurs because of binding to homologous structures in human mitochondrial 16S sRNA, resulting in reduced synthesis of key components of respiratory chain complexes I, III, IV and ATP synthase, and impairment of oxidative phosphorylation. These domains in the mitochondrial PTC are partly encoded by mitochondrial DNA, and mutations in the 16S rRNA gene (A2706G and G3010A) have been associated with linezolid-induced mitochondrial toxicity. Complex II is encoded in the nucleus and synthesised by cytoplasmic ribosomes, and thus not affected by linezolid exposure [77, 137, 182].

Multiple lines of evidence support a causal relationship between linezolid's activity and mitochondrial toxicity [77, 183, 184], which shows a dose-response effect [90] and is time-dependent [77, 185]. The degree of mitochondrial protein inhibition is more pronounced with increasing oxazolidinone antibacterial potency [90]. Using complex II (which is synthesized by cytoplasmic ribosomes) as a control, De Vriese and colleagues demonstrated significantly reduced activity of complex IV (cytochrome oxidase, a critical

component the electron transfer chain) in tissue samples from a patient with hyperlactatemia and optic neuropathy who had been treated with a prolonged course of linezolid. This selective decrease in complex IV (and other mitochondrial-derived respiratory chain complexes) was replicated in a rat model where the effect was time- and dose-dependent. This study showed a specific impairment in mitochondrial protein synthesis, as mitochondrial mass appeared to be preserved, reflected in similar citrate synthase activity to controls [77]. Other studies have shown a reduced ratio of complex IV to complex II in the peripheral blood mononuclear cells from patients who had developed symptomatic hyperlactatemia during prolonged linezolid therapy [183, 184], and that mitochondrial protein synthesis recovers within two weeks after withdrawal of linezolid [184]. A direct link between oxazolidinone-induced inhibition of mitochondrial protein synthesis and inhibition of cell proliferation has also been demonstrated, clarifying the role of linezolid in myelosuppression [185].

Of clinical importance, the linezolid concentration that inhibited 50% of mitochondrial protein synthesis (IC_{50}) in the tissue of experimental animals was 3.37 – 5.26 mg/L [90], which overlaps with the range of serum trough concentrations after a standard 600 mg 12-hourly dose used in adults with Gram-positive infections (1 – 6 mg/L) [184]. In a hollow fibre infection model, even a reduced dose equivalent to 600 mg daily led to major reductions in complex IV [105], implying a risk of toxicity even with the use of this dose, particularly with the longer courses required for tuberculosis.

Mitochondrial toxicogenomics

The homology of the conserved regions in the PTC of mitochondrial ribosomal RNA with those found in the prokaryote determines the risk of toxicity by enhancing antibiotic mitochondrial ribosomal binding. For example, aminoglycoside antibiotics are associated with a higher risk of ototoxicity in individuals with A1555G or C1494T mutations in the mitochondrial 12S rRNA gene. These polymorphisms are at sites which encode key functional regions of mitochondrial 12S rRNA, and result in alterations at these sites which are homologous to bacterial 30S rRNA aminoglycoside targets [186].

Although there is a direct relationship between linezolid exposure and the development of mitochondrial toxicity, its incidence and severity is variable, suggesting the possibility of a genetic predisposition. The 16S RNA mitochondrial ribosomal subunit is encoded

by mitochondrial DNA (mtDNA), and mutations in this region could confer susceptibility to linezolid toxicity. Single nucleotide polymorphisms (SNPs) in the 16S rRNA gene (A2706G and G3010A) have been described in case reports of patients with linezolid-induced lactic acidosis [82, 187], but these SNPs are relatively frequent in the general population [188] and were not associated with adverse events in a cohort of 38 Korean patients given linezolid for XDR tuberculosis [83]. However, the substitutions are situated very close to the PTC, and the A2706G mutation lies in an exposed position on the 16S rRNA that could enhance its interaction with linezolid [82]. These factors imply a potential relationship between these SNPs and linezolid inhibition of mitochondrial protein synthesis [78]. Genetic associations with adverse events should be explored on a larger scale, particularly in African populations, who may have different population mtDNA polymorphisms affecting predisposition to linezolid toxicity.

Clinical impact of mitochondrial toxicity

The effect of cumulative linezolid dose on mitochondrial toxicity has been seen in clinical practice, where serious adverse events such as myelosuppression, optic and peripheral neuropathy, and lactic acidosis, occur more commonly with linezolid use beyond 28 days, which is the FDA-approved maximum duration [79]. The duration of linezolid therapy in drug resistant tuberculosis usually exceeds 6 months (and may be continued for up to 24 months); this prolonged administration frequently leads to adverse events requiring treatment discontinuation or dose reduction.

In the most recent systematic review of linezolid use in drug resistant tuberculosis [81], which included 367 patients from 15 studies, 55% experienced adverse events and over a third (35%, 95% CI 22 – 47) suffered major toxicities requiring discontinuation of therapy. Even at daily doses ≤ 600 mg, major adverse events occurred in 25%. The most common adverse events were peripheral neuropathy and anaemia, occurring in 31% (95% CI 19 – 42) and 25% (95% CI 15 – 34) respectively. Fortunately, these effects are usually reversible on discontinuing linezolid or with dose reductions, but cases of fatal lactic acidosis [189], and persistent peripheral neuropathy [190, 191] have been described. Neurological complications appear to be duration-dependent [192], usually developing after 2 months on therapy, even with lower doses, while haematological toxicity usually occurs within the first 2 months of therapy, is reversible, and is associated with higher doses [63, 81, 174]. The timing of mitochondrial toxicity thus has

important implications for designing linezolid treatment regimens, emphasizing the need to evaluate the efficacy of shorter courses.

As noted above, there are limited data on the use of linezolid for drug-resistant tuberculosis in HIV-infected patients. In a retrospective cohort study involving 34 patients with drug-resistant tuberculosis from Cape Town and Mumbai, there was a trend towards more frequent linezolid-related adverse events amongst the 17 HIV-infected patients (HR 2.9, 95% CI 0.9 – 9.4) [175]. Potential contributing factors for this increased toxicity include (1) overlapping toxicity with ART, particularly the non-nucleoside reverse transcriptase inhibitors, which can cause mitochondrial toxicity by inhibiting gamma polymerase which controls mtDNA replication [193], (2) pre-existing HIV-associated peripheral neuropathy and anaemia, and (3) direct mitochondrial dysfunction from HIV itself [194].

There is a direct correlation between linezolid dose, trough concentrations and the development of clinical toxicity [63, 64, 83, 110]. In the Korean RCT [83], patients who were randomized to continue the 600 mg linezolid dose had significantly higher toxicity risk and earlier occurrence of adverse events compared to those changed to 300 mg (HR 3.1, 95% CI 1.23 – 7.86). Although linezolid use was associated with a decline in mitochondrial translational capacity (measured by cytochrome c oxidase/citrate synthase ratio) in all participants, the effect was significantly greater in those continuing to take the 600 mg dose. There was a linear relationship between dose and linezolid trough concentrations, with a 2-fold increase in risk of clinical toxicity for every 1 mg/L increase in trough concentration [83]. Because of the shared mechanisms of anti-mycobacterial activity and mitochondrial toxicity, attempts to increase potency with dose increase will predictably be accompanied by greater toxicity, but it is unclear what impact dose reductions for toxicity have on efficacy or the risk of linezolid resistance.

Resistance

Mutational linezolid resistance in Gram-positive bacteria is difficult to select *in vitro* [137]. Although resistant strains of Gram-positive pathogens have emerged during therapy, surveillance programs have demonstrated sustained susceptibility, with linezolid resistance prevalence consistently below 0.5% and no evidence of ‘MIC creep’ [195]. Linezolid therefore appears to have a relatively high barrier to resistance; possible

explanations for this include the fact that it is a chemotherapeutic agent rather than an antibiotic, the lack of cross-resistance with other ribosomal agents, and the presence of multiple copies of genes that encode its primary binding site [196].

Resistant *M tuberculosis* strains are also not easily generated *in vitro* after exposure to sub-inhibitory concentrations of linezolid [117, 169]. However, the first clinical strains with linezolid resistance were reported soon after its introduction as an anti-tuberculosis agent, as early as 2007, from 4 patients with MDR tuberculosis and prior linezolid exposure [111]. In these strains with MICs ranging from 4 to 8 mg/L, no mutations were identified in 23S rRNA (*rrl)* or other putative target genes coding for ribosomal proteins surrounding the binding site (L4 and L22) or involved in the protein initiation complex (23S rRNA methyltransferase). But, when the same investigators sequenced the 23S rRNA gene from 10 linezolid-resistant strains selected *in vitro*, they found mutations resulting in G $\rightarrow$ T base pair exchanges at nucleotide position 2061 in four strains with MICs of 32 mg/L and at position 2576 in one strain with an MIC of 16 mg/L. The association of these mutations with reduced linezolid potency is unsurprising given their proximity to the linezolid PTC binding site [197], and their association with such elevated MICs would likely to contribute to treatment failure. In contrast, the remaining 5 strains with lower MIC values of between 4 and 8 µg/mL showed no mutations in the 23S rRNA gene [117]. The molecular mechanism that underlies this low-level resistant phenotype was subsequently demonstrated to be a mutation in the *rplC* gene (T460C) from both *in vitro* selected mutants and clinical isolates with MICs of 2 to 16 mg/L [122]. The position of the *rplC* T460C mutation corresponds with a linezolid binding site on part of the ribosomal L3 protein that extends into the PTC, and has been associated with linezolid resistance in Gram-positive bacteria [197]. Its role in *M tuberculosis* resistance to oxazolidinones was recently confirmed by demonstrating increased MICs of linezolid and another oxazolidinone, sutezolid, against recombinant *M tuberculosis* strains with overexpressed *rplC* T460C mutations [124]. The emergence of *rplC* T460C during therapy in patients with acquired linezolid resistance [122] is further evidence that it contributes to treatment failure. Thus, it appears that mutations in the 23S rRNA gene mediate higher levels of resistance to linezolid in *M tuberculosis*, and that *rplC* mutations are responsible for lower-level resistance. However, 23S rRNA mutations have been found in clinical isolates with lower MIC values [63], raising the possibility of an associated fitness cost.

Only five published studies [63, 111, 117, 121, 122], summarized in Table 1, have evaluated the mechanisms of linezolid resistance in *M tuberculosis*, and there may be additional as yet unidentified mutations or non-ribosomal mechanisms that account for resistance. Highlighting this point, in a recent laboratory surveillance study, 12 out of 17 (70%) linezolid-resistant strains (including those with MICs of 16 and 32 mg/L) lacked mutations in targeted genes (*rrl*, *rplC* and *rplD*) [121]. The inherent resistance of Gram-negative bacteria to linezolid results from active extrusion by an efflux pump, and this may be a possible non-ribosomal resistance mechanism in *M tuberculosis* strains with moderately elevated MICs but no mutations in ribosomal 23S RNA or L3 [111, 198, 199]. Another non-ribosomal mechanism may be related to cell wall thickness in XDR tuberculosis strains, reducing permeability to linezolid [200], but these potential mechanisms require further investigation.

The risk and frequency of linezolid resistance in tuberculosis treatment is largely unknown. In the Chinese laboratory-based study cited above [121], 10% of screened isolates had elevated linezolid MICs, but the drug exposures and clinical details of these patients were not reported, and so the true denominator is ill defined. In this study linezolid resistance was significantly associated with the Beijing genotype (the dominant circulating strain in high tuberculosis burden countries [32, 201]) and was more frequent among XDR isolates. Of concern, a trend of increasing linezolid MICs (0.5 to 2 mg/L) amongst selected clinical MDR strains has been seen in China since 1995, even from patients with no prior linezolid exposure [202]. In the Korean RCT [63], where linezolid was added onto a failing regimen and this constituted effective ‘monotherapy,’ 4 participants (11%) developed acquired linezolid resistance (MIC range 2 to 16 mg/L) with associated *rrl* and *rplC* mutations. Three of these participants had been randomized to the reduced 300 mg dose, and trough concentrations of linezolid were lower than the isolate MICs in 9 of the 16 participants assigned this group. By contrast, trough concentrations of linezolid were above the isolate MICs for almost all participants taking a 600 mg daily dose.

Table 1. Mutations found in linezolid-resistant *M tuberculosis* strains.

Author, year	Description	Gene(s) sequenced	Mutations	MIC range (μg/mL)
Richter 2007 [111]	4/210 clinical isolates	23S rRNA (*rrl*), L4, L22, 23S rRNA methyltransferase	None found	4 – 8
Hillemann 2008 [117]	10 isolates selected *in vitro*	23S rRNA	G2061T	32
			G2576T	16
Beckert 2012 [122]	Clinical and *in vitro* isolates	L3 (*rplC)*	T460C	4 – 16
Lee 2012 [63]	4/38 isolates from a clinical trial	23S rRNA (*rrl*)	G2447T	16
			G2576T	4
		L3 (*rplC)*	T460C	2 – 4
Zhang 2014 [121]	17/ 158 clinical isolates	23S rRNA (*rrl*)	G2061T	32
		L3 (*rplC)*	T460C	16 - 32

These observations suggest a relationship between dose, trough concentrations, and risk of resistance, which has implications for designing linezolid-based regimens for tuberculosis. There have been conflicting findings regarding the impact of dosing regimens on resistance suppression. Supporting once-daily dosing are findings from a pharmacodynamic (PD) model of *B anthracis*, where this approach both optimized bacterial kill and minimized the probability of resistance amplification compared with 12-hourly regimens [203]. However, in a hollow-fibre *M tuberculosis* infection model the relationship between linezolid exposure and the size of resistant sub-populations followed an “inverted U-shaped” curve, where resistant sub-populations emerged rapidly with increasing doses, reaching a peak at 600 mg daily dose equivalent exposures [105]. Although the standard 600 mg daily dose for tuberculosis is likely to achieve concentrations above the published mutant prevention concentration (MPC) of 1.2 mg/L [204], the hollow fiber model raises concerns that further dose reductions (necessitated by toxicity issues) may select out resistant strains during tuberculosis treatment, particularly in the context of a weak background regimen. Indeed, in the two published reports that have provided clinical data on linezolid resistance, all patients were treated with background regimens of questionable efficacy [63, 111].

What is the lowest effective dose and shortest duration of linezolid for tuberculosis that prevents the emergence of resistance?

Understanding the correlation between PK parameters and clinical outcomes is necessary to determine the optimal dose for linezolid, particularly as a component of multidrug tuberculosis regimens where PK variability of drugs is frequently high, and individuals with lower exposures are at risk of the emergence of drug resistance and treatment failure [205, 206]. Linezolid activity is both time- and concentration-dependent, and recommended target PK parameters for efficacy in Gram-positive bacterial infections are a free area under the 24-hour concentration–time curve to MIC (AUC_{0-24}/MIC) ratio > 100 and a time above MIC (T > MIC) of more than 85% [102, 207-209]. Besides a murine study showing that AUC_{0-24}/MIC is the best PD predictor of efficacy in tuberculosis [72], PK/PD targets for linezolid in tuberculosis treatment have not been adequately established, and current dosing practices are not based on prospectively collected data which integrate drug exposures derived from PK/PD models with clinical outcomes data or MICs from clinical isolates. What has been clearly established, though, is that linezolid's shared mechanism of anti-mycobacterial activity and mitochondrial toxicity leads to dose- and duration-related adverse events, which is a major limitation of linezolid use in tuberculosis treatment. This has forced clinicians to use lower doses of linezolid in tuberculosis treatment, but in the absence of well-defined PK targets for efficacy, the impact of this on efficacy and resistance suppression is unclear.

Linezolid PK/PD targets for tuberculosis have been extrapolated from its use in Gram-positive infections and inferred from *in vitro* and small clinical PK/PD studies in tuberculosis. It has been estimated that when treating *M tuberculosis* strains within the wild-type MIC distribution, a half-standard linezolid dose of 600 mg daily would translate into AUC/MIC ratios exceeding 109, achieving the PD target for *S. aureus* [167]. The widespread adoption of the 600 mg daily dose in tuberculosis treatment is based largely on this estimation, plus other limited clinical data [210], and on the assumption that PD efficacy parameters are similar in *M tuberculosis*.

However, as described above, even this dose is associated with substantial toxicity, and it may not achieve optimal efficacy. In an EBA study involving patients with drug-susceptible tuberculosis, although linezolid 600 mg daily exhibited comparable *M*

tuberculosis killing to twice daily dosing, the daily dosing schedule resulted in lower exposures and resulted in T > MIC values below Gram-positive targets [73]. Furthermore, the traditional PK/PD parameters used for Gram-positive infections (AUC/MIC and T>MIC) may not be ideal as linezolid targets in tuberculosis treatment. A hollow fibre model suggested that trough concentrations (trough/MIC) may be a key efficacy parameter by showing that a 300 mg 12-hourly equivalent had better kill rates than the 600 mg daily dose equivalent [105]. And, in a phase I trial comparing the bactericidal activity of linezolid with sutezolid, the maximal bactericidal effect occurred at peak concentrations above 2 times the MIC, supporting a concentration-dependent effect. Using this concentration-activity relationship, the authors predicted that a 600 mg daily dose of linezolid would achieve levels associated with peak bactericidal effect for only ~60% of the dosing interval [211].

In attempts to further minimize toxicity, daily linezolid doses of 300 mg have been used, but this appears to lead to an even greater loss of potency and efficacy *in vitro*. In the hollow fibre experiment referred to above, the 342 mg linezolid dose equivalent (-1 standard deviation below a mean 600 mg dose equivalent) killed substantially less than exposure to the 600 mg dose equivalent [105]. Similarly, a modelling study (using PK data from adults without tuberculosis) estimated that for *M tuberculosis* isolates at the suggested susceptibility breakpoint (1 mg/L) a 300 mg daily linezolid dose would result in lower median T > MIC values than 600 mg daily, and that both would fall below the recommended range for Gram-positive infections (median 56% [IQR 36 – 100] vs 78% [51 – 100], respectively) [212].

A limited number of studies have assessed clinical outcomes with various linezolid dosing regimens, and these are summarized in Table 2. To date, clinical data for the 600 mg daily linezolid dose have been reported for only 202 patients with tuberculosis [81, 174-176, 213, 214]. The most recent systematic review (which included efficacy data from just 117 patients) found differences in culture conversion (84.6% versus 66%, P = 0.771) or cure (64.9% versus 53.1%, P = 0.066) between those taking ≤ 600 mg and those on total daily doses > 600 mg, but this was not statistically significant [81]. More treatment failure and deaths occurred in the lower dose group, although the reasons for this are unclear and may be confounded by patient factors such as HIV

status, disease severity, and degree of drug resistance [81]. Two studies involving 67 patients have specifically evaluated a 300 mg daily dose [63, 215].

Table 2: Clinical outcomes of different linezolid dosing regimens for drug resistant tuberculosis.

	300 mg daily		**600 mg daily**					**>600mg daily**
Author, year	Koh, 2012 [215]	Lee, 2012 [63]	Zhang, 2015 [81]	Zang, 2014 [213]	Tang, 2015 [174]	Hughes, 2015 [175]	Liu, 2015 [216]	Zhang, 2015 [81]
Denominator	51	See legend[c]	See legend[d]	15	33	34	16	See legend[f]
Proportion XDR (%)	51	100	53.6	100	100	50	100	41.2
Median time to culture conversion (days)	55	75	57.7	NR	NR	NR	NR	106.6
Proportion with culture conversion (%)	78.4	87	90.3[e]	60	78.8	50 (n = 28)	87.5	86
Cure[a] (%)	73.3	71	57.6[e]	NR	51.5	NR	84.6 (n = 13)	53.1
Major adverse event[b] (%)	27	18	25[e]	NR	NR	5.9	12.5	31.9

Table 2. *NR* not reported. [a] According to WHO definition; [b] Requiring interruption/discontinuation of linezolid therapy; [c] 38 patients received linezolid, 16 were initially randomised to 300 mg (13 additional patients had dose reductions to 300 mg during therapy). Data includes all 38 patients who received linezolid, 29 of whom were on the 300 mg dose at some stage during therapy; [d] Systematic review of all published studies of linezolid use in TB treatment. Denominators for culture conversion 117, cure 111, and adverse events 151; [e] Calculated without data from Koh, et al; [f] Systematic review of all published studies of linezolid use in TB treatment. Denominators for culture conversion 121, cure 128, and adverse events 216

Unfortunately, in one of these, a clinical trial, the efficacy outcomes of the subgroup of patients randomized to reduce dose to 300 mg/day were not reported, and these could be affected by the prior exposure to 600 mg daily in all participants. The retrospective

study by Koh and colleagues included 51 Korean HIV-negative drug resistant tuberculosis (50% XDR) patients who were failing therapy and provided linezolid 300 mg daily from the start of treatment. Despite being added to a weak background regimen (containing only 2 other agents to which the isolates were susceptible) linezolid 300 mg/day resulted in culture conversion after a median duration of 55 days and favourable outcomes (cure or completion of treatment after culture conversion) in 78%. Disappointingly, major adverse events requiring discontinuation of linezolid still occurred in 27%, but withdrawal of linezolid did not influence treatment success rates.

These observations support the possibility of limiting the duration of higher dose linezolid therapy to reduce the cumulative exposure and minimize toxicity; this requires formal evaluation. A recent mouse model provided additional support for this strategy by showing that the sterilizing activity achieved by linezolid-containing regimens was not reduced when the duration of high dose therapy was shortened to 1 month [173]. An important consideration, however, is the impact of lower doses on linezolid resistance, and in the modelling study discussed above, a daily dose of 300 mg was predicted to achieve a median T > MPC under 60% [212].

Other dosing approaches have been tried, including intermittent dosing and a 300 mg twice daily regimen. The latter achieves favourable efficacy exposures [210, 212], but mitochondrial protein synthesis appears to be sensitive to more frequent dosing intervals [105], and trough concentrations exceed the IC_{50} for mitochondrial toxicity more than 50% of the time [212]. Once-daily dosing may thus be preferable in terms of enhancing treatment adherence and lowering the risk of linezolid-induced toxicity.

A Chinese study prospectively evaluated an intermittent dosing schedule of linezolid in 10 patients with pre-XDR and XDR tuberculosis, most of whom had failed treatment. Linezolid was initially dosed at 800 mg daily for up to 4 months and switched to 1,200 mg thrice weekly after culture conversion or intolerance; the dose was further reduced to 600 mg thrice weekly in 6 patients who developed toxicity. Adverse events were observed less frequently during intermittent dosing, and all patients achieved cure by a median of 18 months. During the 1,200 mg thrice weekly schedule, serum linezolid concentrations were below the lower IC_{50} limit for mitochondrial toxicity for 34 – 62% of the dosing time interval, above the MIC susceptibility breakpoint for 60 – 100%, and

over 4-fold the MPC value for 34 – 61% [149]. These findings suggest a time-dependent effect of linezolid in tuberculosis treatment and lend support to the use of a higher dose 'induction' followed by dose reduction after culture conversion. Intermittent dosing during this 'maintenance' phase may reduce toxicity by allowing periods where there may be minimal or no exposure of mitochondria to drug.

These dosing strategies need to be confirmed by larger studies that are able to define the optimal targets within linezolid's narrow therapeutic window: In the Korean RCT, adverse events occurred in all patients with mean linezolid trough concentrations >2 μg/mL [83], and on the other hand, exposures ≥ 2 μg/mL were required to achieve maximal bactericidal activity in a whole-blood human infection model [217] that correlates with 2-month culture status [218] (but not necessarily relapse-free cure). An optimal linezolid dosing regimen that achieves consistent exposures above the MIC and MPC for the *M tuberculosis* wild type distribution, and remains below the IC_{50} for mitochondrial toxicity, may be unachievable, but the clinical utility and safety of linezolid could possibly be optimized with better defined PK/PD targets and by the use of therapeutic drug monitoring (TDM).

Therapeutic drug monitoring for linezolid

TDM may be an important tool in the management of tuberculosis because of the high degree of PK variability of many anti-tuberculosis agents (including linezolid), and the potential for treatment failure and resistance for patients at the lower end of the PK distribution [205, 206]. Evidence is emerging that TDM has a role in reducing adverse events and improving outcomes in patients who have poor response to therapy in drug-susceptible tuberculosis [219], and it is being used routinely in some centres in the management of drug-resistant tuberculosis [146].

The use of TDM for linezolid is appealing because of its narrow therapeutic range and wide inter-individual variability [220]. Because linezolid trough concentrations correlate linearly with AUC [100, 220] and clinical toxicity [83], TDM could be used to guide dosing if the PK relationship with toxicity and efficacy in tuberculosis is better defined. TDM-guided dose adjustment has been used successfully to predict [221, 222] and reduce [109] thrombocytopenia in patients on long-term linezolid for Gram-positive infections. There have been no published reports on the impact of linezolid TDM on clinical

outcomes in tuberculosis treatment, but two important factors support the feasibility of this practice. First, linezolid concentrations in oral fluid have an excellent correlation with serum AUC_{0-12} and AUC_{0-24}/MIC values [223], and second, dried blood spot analysis [224] which could be performed in resource-limited settings shows good agreement with linezolid plasma concentrations [225]. A potential limitation of the use of TDM is the non-linearity between linezolid dose and exposure [210, 226]; more PK/PD data are needed to inform predictive models for individualized dosing to support routine use of TDM in drug-resistant tuberculosis therapy.

Other oxazolidinones

The limitations of linezolid, together with the recognition of the anti-mycobacterial activity of oxazolidinones as a class, have spurred the development of more potent and less toxic analogues. To date, two other oxazolidinones, sutezolid and AZD5847 (posizolid), have been clinically assessed for anti-mycobacterial activity. Although AZD5847 demonstrated superior bactericidal activity against *M tuberculosis* compared to linezolid *in vitro* [123] and a significant decline in *M tuberculosis* quantitative sputum cultures in a phase 2a trial [227], a substantial number of patients treated with AZD5847 experienced severe or life-threatening hepatic and haematological events, halting further development [228]. Sutezolid, on the other hand, may have a promising future role in tuberculosis therapy. Despite having a similar MICs to linezolid against *M tuberculosis*, it is significantly more potent *in vitro* [229] and in mouse models [70], even at lower exposures [230], suggesting that lower dosing may be possible with lower risk of toxicity. Sutezolid also has superior bactericidal activity against non-replicating *M tuberculosis* bacilli [171], and its use greatly enhances the bactericidal and sterilizing effect of combination therapy in mice [173]. In a phase 1 study, sutezolid showed superior *ex vivo* anti-mycobacterial activity compared to linezolid, independent of peak concentrations [211], and has also demonstrated EBA in patients with drug-sensitive pulmonary tuberculosis [231]. It was safe and well-tolerated in these human studies, and importantly, no clinically significant haematological abnormalities were detected after 28 days of therapy at a dose of 600 mg twice daily [232]. This favourable toxicity profile is likely a result of mean concentrations remaining below the IC_{50} for mitochondrial toxicity across a range of doses up to that dose [232], but may also be due to improved anti-mycobacterial selectivity. Despite these apparent advantages over linezolid, there appear to be no planned trials of sutezolid 2 years after publication of

the single phase 2a trial, creating uncertainties about its future clinical development. Other highly selective oxazolidinones are at various stages in the development pipeline.

Expert commentary

Linezolid is the only licensed oxazolidinone that is recommended in patients with drug-resistant tuberculosis. It has substantial potency against *M tuberculosis* and there is a growing evidence base for its efficacy in drug resistant tuberculosis treatment. Until recently there have been few therapeutic options for pre-XDR and XDR tuberculosis, and linezolid has become an essential addition to treatment regimens for these infections [170, 175].

However, no adequately powered clinical PK/PD studies have been conducted to address the uncertainties of linezolid dosing for tuberculosis. The available PK/PD data of different dosing regimens for tuberculosis are summarized in Table 3. None of these studies have found associations between PK parameters and clinical efficacy, but all have lacked sufficient power to do so. Although the data from *in vitro* and clinical studies are limited, a number of observations can be made: (1) there is a high degree of PK variability between studies and between patients, (2) there is a non-linear relationship between dose and exposure, but trough concentrations correlate well with toxicity and possible efficacy targets, (3) the 600 mg twice daily dose is likely to achieve PK/PD targets set for Gram-positive infections but results in exposures that carry a high risk for toxicity, (4) the 600 mg daily dose may not reach PK/PD targets in all patients, especially at *M tuberculosis* MICs near the clinical breakpoint of 1 mg/L, and is associated with exposures that may lead to substantial toxicity, and (5) the 300 mg daily dose does not achieve adequate exposures for efficacy and resistance suppression, but has trough concentrations below the IC_{50} for mitochondrial toxicity. The uncertainties regarding the optimal dose of linezolid and use in HIV-associated tuberculosis require further study to support its expanded use.

Table 3: PK/PD data for linezolid from patients with tuberculosis and from a modelling study [212] using PK data from patients without tuberculosis.

Dose	Author, year	*f*AUC$_{0\text{-}24}$ (mg·h/L)	*f*AUC$_{0\text{-}24}$/MIC	T>MIC (%)	C_{min} (mg/L)	[e]*fAUC*/MPC$_{90}$

600 mg 12-hourly	McGee, 2009 [233] (n = 9)	160.7 (134.4–225.8)	243.2 (159.7–283.2)	100 (100 – 100)	NR	133.9
	Alffenaar, 2010 [210] (n = 12)	123.8 (100.9–152.5)[b]	720 (347 – 2880)	NR	4.4 (2.7–7.5)	103.2
	Dietze, 2008 [73] (n = 9)	232.9 (100.8–394.4)	465.8	100	NR	194.1
600 mg daily	McGee, 2009 [233] (n = 10)	66.8 (33.0–99.2)	116.2 (71.0–138.4)	62.8 (54.6–77.0)	NR	55.7
	Dietze, 2008 [73] (n = 10)	96.9 (47.8–143.7)	193.8	NR	NR	80.75
	Lee, 2012 [63] (n = 17)	126.3 (63.7 – 188.6)[c]	252.6[d]	NR	NR	150.3
	Barry, 2014[a] [212]	85.8	171.6[d]	98 (65-100)[d]	NR	71.5
300 mg 12-hourly	Bolhuis, 2013 [154] (n = 7)	63.9 (47.8–83.8)[b]	277 (260–517)	NR	2.2 (1.5–4.2)	53.3
	Alffenaar, 2010 [210] (n = 14)	51.8 (41.8–65.9)[b]	235 (92-829)	NR	1.7 (0.9–2.5)	43.2
300 mg daily	Lee, 2012 [63] (n = 16)	63.8 (33.7–93.9)[c]	127.6[d]	NR	NR	75.9
	Koh, 2009 [91] (n = 10)	NR	NR	NR	2.1 (0.8–3.4)	NR
	Barry, 2014[a] [212]	42.3	84.6[d]	77 (50-100)[d]	NR	35.3

Table 3. Data are median (IQR) unless otherwise specified. *NR* not reported, *AUC* area under the serum concentration-time curve, *MIC* minimum inhibitory concentration, *T* time, C_{min} trough concentration, *MPC* mutant prevention concentration. MICs were median values from study isolates unless otherwise specified. All parameters have been adjusted for free drug concentrations (*f*), assuming linezolid protein binding of 30%. [a] Modelling study, data from non-TB patients; [b] AUC_{0-12};

[c] Mean (SD); [d] Using MIC of 0.5 mg/L (suggested ECOFF) [167]; [e] Calculated values using MPC_{90} 1.2 mg/L [204]

Five-year view

There are several planned or active phase 2/3 trials assessing the role of linezolid in drug resistant tuberculosis [228]; these are summarized in (Table 4). PK/PD studies are essential tools in informing the optimal use of tuberculosis drugs [234, 235], and a feasible approach to generate data that will better define linezolid dosing is to undertake observational PK/PD studies nested in existing trials and large observational cohorts, using the data to model the relationships between dose and exposure, and safety, efficacy, and resistance.

Table 4: Ongoing phase 2 and 3 clinical trials of linezolid for drug resistant tuberculosis.

Trial	Phase	Patients	Design	Primary endpoint
NExT (NCT02454205)	Phase 2-3	MDR-TB, adults n = 300	Open label RCT of an injection-free regimen including linezolid[a] and bedaquiline (plus standard drugs without kanamycin) for 6 – 9 months compared with WHO standard regimen	Favourable outcome at 24 months
Nix-TB (NCT02333799)	Phase 3	MDR- and XDR-TB, adults n = 200	Open label, single arm evaluation of bedaquiline and pretomanid plus linezolid[b] for 6 – 9 months	Bacteriologic or clinical failure at 24 months
endTB (NCT02754765)	Phase 3	MDR-TB, adults n = 750	Open label RCT of 5 all-oral experimental regimens compared with standard of care.	Favourable outcome at 18 months

			Experimental regimens contain bedaquiline and/or delamanid together with 4 companion drugs, including linezolid[c]	
TB-PRACTECAL (NCT02589782)	Phase 2-3	MDR-TB, adults n = 630	Open label RCT comparing 3 novel regimens including bedaquiline, pretomanid, and linezolid[d], plus moxifloxacin or clofazimine for 6 months with WHO standard of care	Culture conversion and discontinuation/death at 8 weeks, unfavourable outcome at 72 weeks
MDR-END (NCT02619994)	Phase 3	MDR-TB, adults n = 238	Open label RCT comparing a 9 – 12 month regimen of delamanid, linezolid[e], levofloxacin, and pyrazinamide with WHO standard or care	Treatment success at 24 months

Table 4. [a] 600 mg daily with a dose reduction to 300 mg daily in the event of toxicity; [b] 600 mg twice daily with a dose reduction in the event of toxicity; [c] Dose not specified; [d] 600 mg daily for 16 weeks then 300 mg daily (or 600 mg x3/week); [e] 600 mg daily for 8 weeks, then 300 mg until the end of treatment

It is likely that future drug regimens for drug resistant tuberculosis will be both shorter and injection-free. The development of potent new anti-tuberculosis agents with unique mechanisms of action has changed the treatment landscape by supporting completely new regimens which are potentially more effective and have a higher barrier to resistance. Linezolid has an important place in these future regimens if the right balance can be achieved between efficacy and mitochondrial toxicity. Its therapeutic window is so narrow that maintaining a single dose throughout therapy is unlikely to be possible

for the majority of patients. With more PK/PD modelling data it may be possible to provide personalized dosing based on weight, renal function, isolate MICs, and trough concentrations. However, this may not be practical in resource-limited settings, where standardized linezolid dosing regimens will need to be continued for cost and programmatic reasons. Based on the limited available data presented in this review, it appears that a strategy for standardized linezolid dosing in the context of new drug regimens may be a short and intensive ‘induction’ course with higher doses (possibly up to 1,200 mg/day) followed by low dose (300 mg/day) or intermittent dosing ‘maintenance’ therapy after culture conversion (or in the event of toxicity) to provide ongoing bacteriostatic support to effective partner drugs in a resistance suppression role. This suggestion, as well as current practice, needs be informed by data from adequately powered PK and outcomes studies.

Finally, it is important to recognize that the high cost of brand-name linezolid represents the major barrier to access. Although there are initiatives in some countries by groups like Médecins Sans Frontières (MSF) to procure affordable generic linezolid, more needs to be done to lower prices and get linezolid onto approved drug lists of national tuberculosis programs [236], so that the battle against drug resistant tuberculosis can be fought with the best available weapons.

CHAPTER 3

Linezolid pharmacokinetics in South African patients with drug resistant tuberculosis and a high prevalence of HIV co-infection

ABSTRACT

WHO recently recommended linezolid should be prioritized in treatment regimens for drug-resistant tuberculosis (TB), but there are limited data on its pharmacokinetics (PK) in this population. We conducted an observational study to explore covariate effects on linezolid PK and to estimate the probability of PK/pharmacodynamic target attainment in South African patients with drug-resistant TB. Consecutive adults on linezolid-based regimens were recruited in Cape Town and underwent intensive PK sampling at steady-state. Non-compartmental analysis was performed. Thirty participants were included: 15 HIV-positive, 26 on the initial dose of 600 mg daily and 4 participants on 300 mg daily after dose reduction for linezolid-related toxicity. There was a negative correlation between body weight and exposure with 17.4% (95% confidence interval [CI], 0.1 to 31.7) decrease in area under the concentration-time curve (AUC_{0-24}) per 10 kg weight increment after adjustment for other covariates. Age was an independent predictor of trough concentration, with an estimated 43.4% (95% CI, 5.9 to 94.2) increase per 10-year increment in age. The standard 600 mg dose achieved the efficacy target of free AUC/minimum inhibitory concentration (MIC) > 119 at wild type MIC values (≤ 0.5 mg/L), but the probability of target attainment dropped to 61.5% (95% CI, 40.6 to 79.8) at the critical concentration of 1 mg/L. When dosed at 600 mg daily, trough concentrations were above the toxicity threshold of 2 mg/L in 57.7% (95% CI, 36.9 to 76.6). This confirms the narrow therapeutic index of linezolid and alternative dosing strategies should be explored.

Introduction

Drug-resistant TB is an ongoing global public health crisis; there were over half a million incident cases in 2017 with a case fatality ratio of approximately 40%, more than double that of drug-sensitive TB [23]. New and repurposed drugs offer the hope of improved outcomes. One such agent, the oxazolidinone linezolid, has an impressive impact on treatment outcomes when added to multidrug regimens for multidrug- (MDR) and extensively drug-resistant (XDR) TB [63, 81]. As a result, linezolid has been promoted to the list of priority 'Group A medicines' in the new WHO antituberculosis drug categorization [237] and is included in the experimental arms of multiple trials of novel regimens for drug-resistant TB. However, linezolid use is limited by dose- and duration-related toxicity, and the optimal dosing strategy that balances efficacy and toxicity is unknown [238].

The pharmacokinetics (PK) that underpins linezolid dosing is poorly defined in patients with TB, particularly at the most commonly used dose of 600 mg daily and amongst patients in sub-Saharan Africa where there is a high burden of HIV co-infection [99]. Understanding linezolid PK is important for several reasons. First, PK variability of antituberculosis agents has been associated with unsuccessful treatment outcomes [206], which may also lead to treatment-emergent drug resistance where drug exposure falls below PK/pharmacodynamic (PD) targets [205]. Population-specific factors, including genetic polymorphisms, may influence drug disposition and drug effects [239], and it is therefore essential to perform PK studies in diverse populations. Second, the myelosuppression and neuropathy associated with linezolid use, which is often treatment-limiting [81], correlates with dose and trough concentrations [83]. Linezolid toxicity may be increased amongst HIV-positive patients [175], which is especially relevant in sub-Saharan Africa where up to 60% of patients with drug-resistant TB are co-infected with HIV. Third, linezolid has limited selectivity for its ribosomal target in bacteria and binds to a homologous site in human mitochondria [77]. Because of these shared linezolid targets in the pathogen and host, there is a narrow therapeutic window for which the optimal PK targets and dose have not been defined [238], but which is likely to be sensitive to PK variability. Finally, efficacy targets of antituberculosis drugs are influenced by minimum inhibitory concentration (MIC) distributions for *M tuberculosis*, but there are limited data on linezolid MICs in populations with drug-resistant TB [240]. Applying observed linezolid drug exposures to putative PK/PD

parameters for efficacy and toxicity may inform policy decisions around dose optimization until more robust clinical targets are defined.

We aimed to describe the PK of linezolid in a population of patients with drug-resistant TB and a high burden of HIV in South Africa. We also explored the effect of key covariates on PK parameters and estimated the probability of PK/PD target attainment corrected for the *M tuberculosis* MIC distribution in this cohort.

Materials and methods

Study population

We conducted a prospective observational PK/PD study of linezolid in adults treated with linezolid containing regimens for drug-resistant TB in South Africa. We enrolled participants from two studies: an observational cohort study of patients with pre-XDR and XDR-TB on bedaquiline containing regimens (PROBeX); and from the intervention arm of an open label clinical trial examining a shortened injection-free regimen for MDR-TB (NExT; ClinicalTrials.gov NCT02454205). The initial dose of linezolid used in both studies was 600 mg daily but was reduced to 300 mg daily in the event of toxicity at the discretion of local clinicians or trial staff. Consecutive participants enrolled in the intervention arm of the NExT trial and those receiving linezolid as part of standard of care in PROBeX were approached to provide informed consent for intensive PK sampling. Eligible participants were over the age of 18 years, had a known HIV test result, and had culture-confirmed drug-resistant TB. Most of the participants in PROBeX were inpatients at the time of the intensive sampling visit, and all of the NExT participants attended as outpatients.

The study was approved by the ethics committees at the University of Cape Town (refs 264/2015 and 920/2015) and Albert Einstein College of Medicine (ref 2014-4348).

Data collection

Participants underwent PK sampling on a single occasion pre-dose and at 1, 2, 3, 4, 5, 6, and 24 hours after a standardized meal and observed linezolid administration. Some participants in the PROBeX cohort had an additional sample taken at 8 and 48 hours as part of other study procedures. The sampling visit was scheduled at Month 2 of linezolid treatment and was thus performed at steady-state. Blood draws were done

through a peripheral intravenous catheter placed for the duration of the first day of the visit. Samples were collected into 10 mL K3EDTA Vacutainer tubes and centrifuged (1,500 x *g* for 10 minutes) within 30 minutes of collection. At least 1.5 mL of plasma was pipetted into polypropylene tubes and immediately frozen at -80°C. Linezolid concentrations were measured in Division of Clinical Pharmacology at the University of Cape Town using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Using a deuterated internal standard, the LC-MS/MS method for linezolid was validated over a calibration range of 0.100 mg/L to 30 mg/L. Over the period of sample analysis (n = 8 batches), a mean percentage accuracy of 98.8 was achieved, with a mean precision of 5.93 (%CV).

Because the 24-hour dose was unobserved and may have been administered prior to the 24-hour sample, concentration-time profiles were inspected for each subject to compare pre-dose and 24-hour concentrations. The 24-hour concentration was considered highly unlikely to represent the true trough value where it exceeded the pre-dose concentration and was > 50% of the concentration at the prior sampling time point (6- or 8-hours). This was based on the published elimination half-life of linezolid of ~6 hours [210, 241], and the assumption that the 24-hour concentration would therefore fall below the 6- or 8-hour concentration in the absence of additional dosing. In these cases, the 24-hour concentration was imputed from either the pre-dose concentration or the mean of the pre-dose and 48-hour concentrations where available (and when the 48-hour concentration satisfied the same criteria in relation to the pre-dose value). Pre-dose concentrations reported as below the limit of assay quantification (BLQ) were imputed as 50% of the lower limit of detection (i.e. 0.05 mg/L), unless there was a history of missed doses prior to the PK visit, in which cases BLQ was replaced by a value of '0.'

Demographic and clinical data were collected from participants at the time of the PK visit, as well as from other visits as part of the parent studies. Data included HIV status, linezolid dose and duration, concomitant antituberculosis drugs and antiretrovirals, and most recent serum creatinine. Timing of administration of linezolid and other antituberculosis drugs was recorded.

Linezolid MIC testing was performed on *M tuberculosis* isolates collected at the time of entry into the parent studies using the mycobacterial growth indicator tube (MGIT) system and continuous growth monitoring with Epicenter software [130]. Dilutions ranged from 0.25 mg/L to 2 mg/L based on the epidemiological cut off (ECOFF) value of 0.5 mg/L [167] and the critical concentration of 1 mg/L [240].

Analysis

Demographic and clinical characteristics were summarized and compared using the Wilcoxon rank-sum test for continuous variables and χ^2 test for dichotomous variables. Non-compartmental analysis was used to estimate linezolid PK parameters from observed concentrations. The area under the concentration-time curve over the 24-hour dosing period ($AUC_{0\text{-}24}$) was computed using the cubic splines method. The trough concentration was defined as the plasma concentration 24 hours after observed intake (actual or imputed as described above). The elimination rate constant (k_e) was assessed by linear regression analysis of the last three concentrations in the terminal log-linear period. The apparent clearance of the drug (CL/F) and the volume of distribution after oral administration (Vd/F) were calculated using standard equations.

We performed linear regression to explore associations between clinically relevant covariates and linezolid exposure. $AUC_{0\text{-}24}$ and trough concentrations were log-transformed and regressed versus weight, age, sex, ethnicity, HIV status, estimated creatine clearance (calculated using the Cockcroft-Gault formula), and concurrent use of ritonavir-boosted lopinavir. This latter parameter was included to explore a possible drug-drug interaction with linezolid, which may be a substrate of the drug transporter P-glycoprotein [154] that is inhibited by HIV protease inhibitors. Parameters with a P value < 0.5 were retained in the multivariable model, using a backward stepwise approach. Regression coefficients were exponentiated and transformed into a value reflecting percentage change ($(e^{\beta} - 1)\cdot 100$) for ease of interpretation.

The PK/PD target for efficacy was defined as free $AUC_{0\text{-}24}$/MIC (*f*AUC/MIC) of 119, based on findings from a hollow fiber infection model [107]. Protein binding of 30% was used to calculate *f*AUC [241]. The PK/PD parameter for toxicity was a trough concentration of 2 mg/L, based on clinical data showing increased mitochondrial and clinical linezolid toxicity above this threshold [83]. The probability of target attainment

was calculated as the proportion of subjects with PK exposures above the efficacy and toxicity targets. Probability distributions were constructed using kernel densities of PK parameters, stratified by MIC. Statistical analysis, including non-compartmental analysis, was performed using Stata version 14.2 (StataCorp).

Results

Study population

Thirty-eight participants were screened between June 2016 and April 2018, and 30 underwent intensive PK sampling. Reasons for exclusion were discontinuation of linezolid prior to the sampling visit (n = 4), withdrawal of consent (n = 2), loss to follow up (n = 1), and failed intravenous access (n = 1). The demographic and clinical characteristics at the time of linezolid sampling are summarized in Table 1. All participants were ambulant at the time of evaluation, including the 21 participants hospitalised for the PROBeX study. Five participants were on lopinavir-ritonavir-based ART. Four participants were on 300 mg daily after undergoing dose reduction for suspected linezolid-related toxicity, one of whom was switched to the 300 mg dose on the day of the study visit and therefore was not at steady state.

PK parameters

Trough concentrations were imputed for 6 participants due to extreme outlying results from presumed unobserved dosing prior to the 24-hour sample. The pre-dose concentration was BLQ in 4 participants. The full dataset showing original and imputed linezolid concentrations is available in the supplementary material (Table S1), along with the respective concentration-time profiles for each subject (Figures S1a and S1b).

Table 1. Demographic and clinical characteristics.

Variable	**N = 30**
Age, years	33 (27 – 44)
Male sex	19 (63)
Weight, kg	58.5 (49.8 – 67.6)
Height, cm	164.5 (158 – 172)
BMI, kg/m^2	20.2 (18.1 – 25.5)

Ethnicity Black Mixed	 14 (47) 16 (53)
Baseline resistance pattern MDR-TB XDR-TB	 9 (30) 21 (60)
HIV positive Current ART Current LPV/r	15 (50) 15 (100) 5 (33)
Creatinine, μmol/L	65 (53 – 71)
Creatinine clearance, mL/min	116 (103 – 139)
Duration on linezolid, days	59 (55 – 63), range (20 – 95)
Daily dose 600 mg	26 (87)
Dose, mg/kg	10.0 (8.3 – 11.5)

Table 1. Data are median (IQR) or n (%). BMI, body mass index; ART, antiretroviral therapy; LPV/r, lopinavir-ritonavir.

Figure S1a. Individual concentration-time profiles using original data.

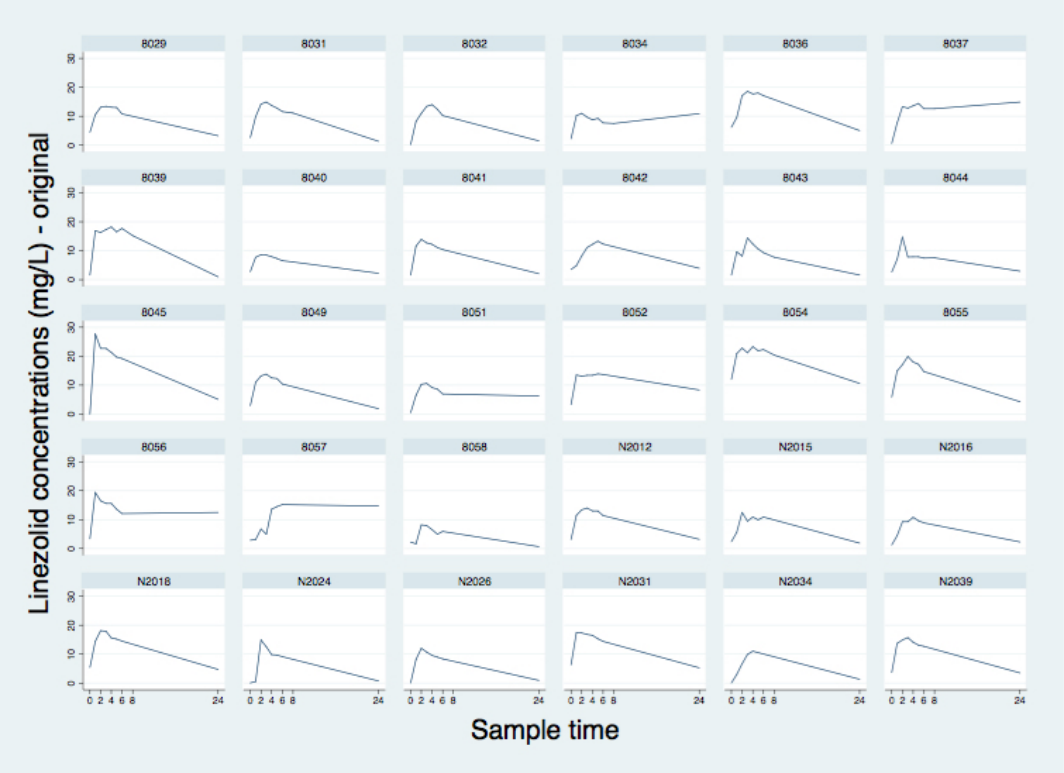


Figure S1b. Individual concentration-time profiles using imputed data.

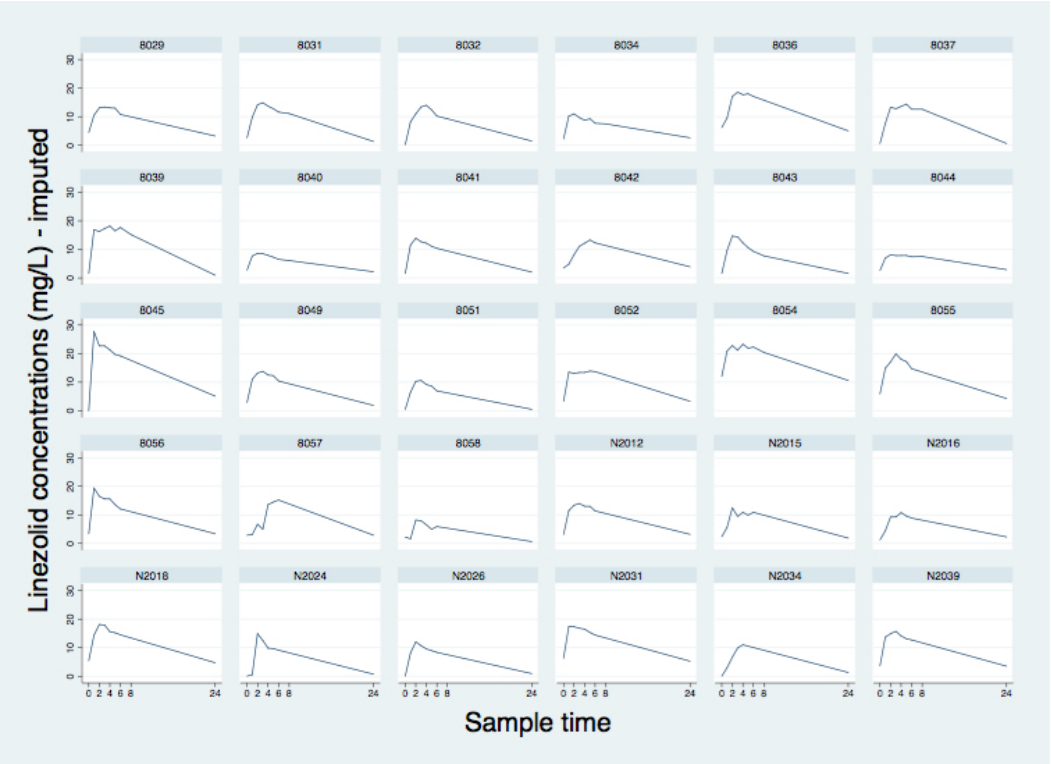


Figure S1a/b. Note that 24-hour concentrations were imputed for the following participants using the approach described in the text of the manuscript: 8034, 8037, 8051, 8052, 8056, 8057.

As shown in Figure 1, concentration-time profiles demonstrated high inter-individual variations in plasma concentrations, with an overall coefficient of variation (%CV) of 40.1%. There was a rapid attainment of peak concentrations, which was similar for both doses, but concentrations at early time points appeared to be highly variable. Table 2 summarizes the estimated PK parameters from observed linezolid concentrations, disaggregated by linezolid dose. Clearance was significantly lower amongst subjects who had undergone dose reduction to 300 mg daily (1.8 L/h (IQR 1.7 to 21) versus 3.1 L/h (IQR 2.4 to 4.3) in those remaining on 600 mg daily; P = 0.012), which resulted in a longer half-life in the 300 mg group. There was a linear correlation between linezolid trough concentrations and $AUC_{0\text{-}24}$; $\rho = 0.5$, P = 0.005 (Figure S2).

Table 2. PK parameters.

Variable	**600 mg (n = 26)**	**300 mg (n = 4)**	**Overall (n = 30)**
$AUC_{0\text{-}24}$, mg·h/L	200.2 (139.9 – 250.8)	165.8 (144.3 – 173.7)	178.9 (139.9 – 244.4)
CV (%)	41.0	13.2	40.1

K_e, h^{-1}	0.08 (0.07 - 0.11)	0.06 (0.06 – 0.09)	0.08 (0.07 – 0.11)
$T_{1/2}$, h	8.4 (6.3 – 9.8)	11.2 (8.6 – 11.9)	9.1 (6.3 – 10.3)
C_{max}, mg/L	14.6 (13.4 – 18.1)	8.4 (8.2 – 9.8)	14.0 (12.0 – 17.4)
T_{max}, h	3 (2 – 4)	2 (2 – 2)	3 (2 – 4)
Trough, mg/L	3.4 (1.6 – 5.1) 74.0	2.4 (1.9 – 2.6) 47.7	2.9 (1.6 – 5.1) 73.0
CL/F, L/h* CV (%)	3.1 (2.4 – 4.3) 69.4	1.8 (1.7 – 2.1) 14.7	2.6 (2.3– 4.1) 71.4
V_d/F, L#	37.8 (24.4 – 54.8)	31.2 (21.6 – 35.9)	36.8 (25.4 – 45.3)

Table 2. Data are median (IQR). AUC_{0-24}, area under the 24-hour concentration-time curve; K_e, elimination constant; $T_{1/2}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time of maximum concentration; Trough, 24-hour/pre-dose concentration; CL/F. clearance; V_d, volume of distribution; CV, coefficient of variation. *Dose/AUC, #CL/k_e

Figure 1. Plasma free concentration-time data for 30 subjects on linezolid.

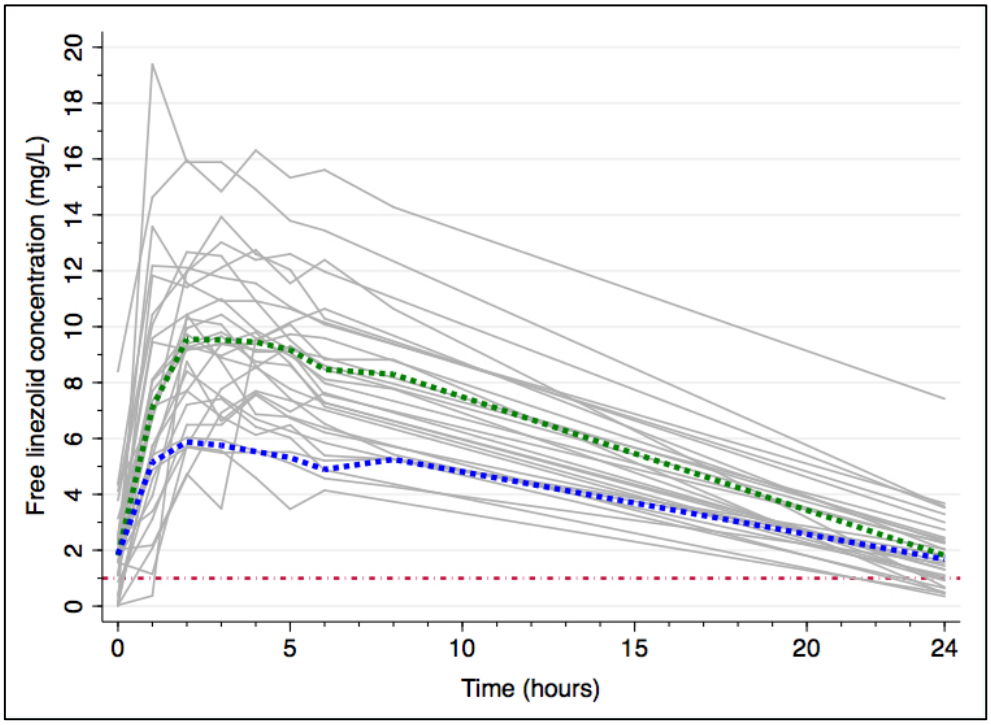


Figure 1. The grey lines represent concentration-time profiles for individual subjects; green dotted line is the median for the 600 mg dose, blue dotted line is the median for the 300 mg dose. The horizontal red line on the y-axis represents the critical concentration of linezolid of *M tuberculosis* (1 mg/L).

Figure S2. Linezolid trough concentrations versus log-AUC0-24.

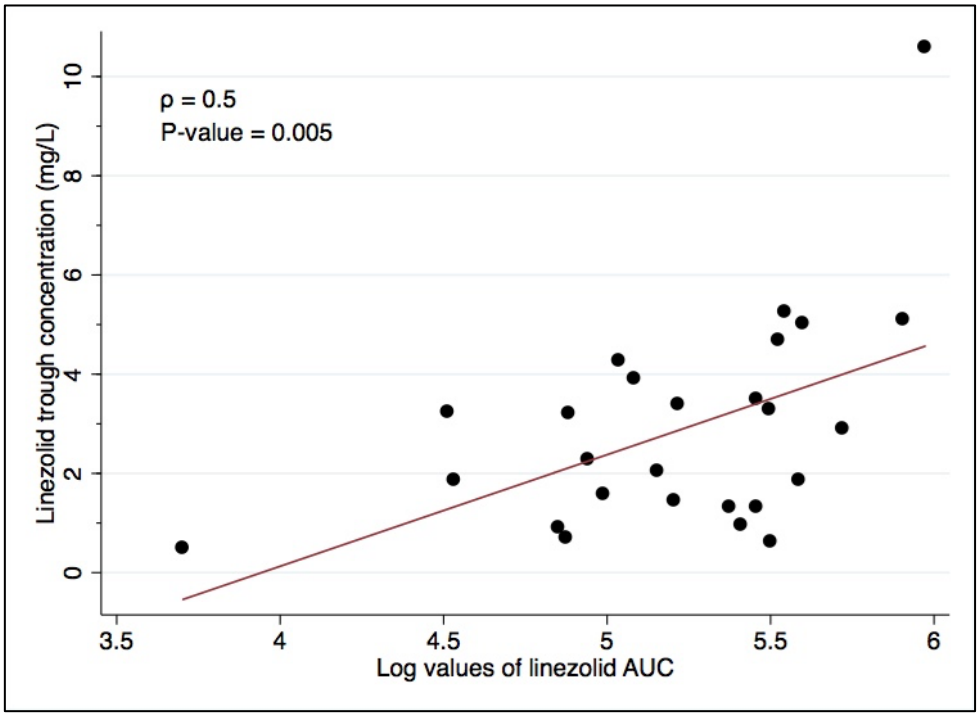


Figure S2. Black circles represent linezolid concentrations of individual subjects; the solid red line is a regression line fitted to the data.

Covariate effects on PK parameters

Linear regression only included participants receiving the 600 mg dose (n = 26) since the sample size of those receiving 300 mg (n = 4) was too small to allow for a meaningful evaluation at that dose. There was no association between HIV infection or the use of lopinavir-ritonavir and linezolid exposure on univariable or multivariable analysis. The final multivariable model described 33% of the variability associated with $AUC_{0\text{-}24}$ (Table 3). After adjustment for age, sex, race, and HIV status, there was a negative correlation between body weight and linezolid exposure, with an estimated 17.4% (95% CI, 0.1 to 31.7) decrease in $AUC_{0\text{-}24}$ per 10 kg increment. Age was significantly associated with higher trough concentrations, and remained an independent predictor on multivariable analysis, with an estimated 43.4% (95% CI, 5.9 to 94.2) increase in trough concentrations per 10-year increment in age (Table 4).

Table 3. Univariable and multivariable linear regression models describing associations between the AUC_{0-24} for linezolid 600 mg daily and selected covariates.

	Univariable		Multivariable	
n = 26	**AUC_{0-24} change* % (95% CI)**	**P value**	**AUC_{0-24} change* % (95% CI)**	**P value**
Male sex	-13.0 (-42.2 – 30.9)	0.488	-24.9 (-49.9 – 12.6)	0.156
Age Per 10-year increase	7.3 (-11.2 – 29.6)	0.452	18.7 (-2.1 – 43.9)	0.078
Black African	9.6 (-26.5 – 63.5)	0.641	-17.3 (-33.1 – 2.2)	0.075
Weight Per 10 kg increase	-11.9 (-25.8 – 4.4)	0.136	-17.4 (-0.1 – -31.7)	0.049
BMI, kg/m^2	-1.6 (-5.9 – 2.9)	0.458		
HIV positive	-14.3 (-42.3 – 27.5)	0.430	-27.2 (-53.5 – 13.8)	0.154
Current LPV/r	-18.9 (-50.9 – 43.1)	0.399		
Dose, mg/kg	7.5 (-2.3 – 18.2)	0.132		
Creatinine clearance, mL/min	-0.5 (-1.1 – 0.1)	0.108		

Table 3. *Percentage change in AUC_{0-24} calculated as $[(e^{\beta} - 1)\cdot 100]$. BMI, body mass index. BMI, body mass index; LPV/r, lopinavir-ritonavir. Variables were excluded from the final multivariable model due to collinearity or as a result of backward elimination after exceeding the P-value inclusion threshold.

Table 4. Univariable and multivariable linear regression models describing associations between linezolid 600 mg daily trough concentrations and selected covariates.

	Univariable		Multivariable	
n = 26	**Trough change % (95% CI)**	**P value**	**Trough change % (95% CI)**	**P value**
Male sex	10.7 (-41.5 – 109.9)	0.744		
Age Per 10-year increase	37.4 (5.4 – 79.2)	0.021	43.4 (5.9 – 94.2)	0.022
Black African	26.1 (-31.9 – 133.3)	0.445	-13.8 (-37.4 – 18.7)	0.346
Weight Per 10 kg increase	9.3 (-17.1 – 43.9)	0.514		
BMI, kg/m^2	1.0 (-5.8 – 8.4)	0.770		
HIV positive	16.1 (-37.7 – 116.2)	0.625	-27.9 (-66.9 – 56.4)	0.389
Current LPV/r	11.5 (-49.4 – 145.5)	0.778	37.1 (-42.9 – 229.6)	0.463
Dose, mg/kg	-4.3 (-17.9 – 11.6)	0.560		
Creatinine clearance, mL/min	-0.2 (-1.2 – 0.8)	0.650		

Table 4. *Percentage change in trough concentrations calculated as $[(e^{\beta} - 1)\cdot 100]$. BMI, body mass index; LPV/r, lopinavir-ritonavir. Variables were excluded from the final multivariable model due to collinearity or as a result of backward elimination after exceeding the P-value inclusion threshold.

Probability of PK/PD target attainment

MIC results were available for the baseline isolates of 16 participants. The median MIC was 0.5 mg/L, range 0.25 to 0.5 mg/L. At this MIC distribution, the probability of efficacy target attainment, defined as a *f*AUC/MIC of 119, was 100% (95% CI, 87 to 100) for the 600 mg dose of linezolid. This finding was consistent after performing a sensitivity analysis using the original outlier trough concentrations. The *f*AUC distributions across four MIC strata are shown in Figure 2. Although the PK/PD target would be achieved in

almost all subjects at the ECOFF value of 0.5 mg/L, only 61.5% (95% CI, 40.6 to 79.8) of patients would exceed an *f*AUC/MIC of 119 at the critical concentration of 1.0 mg/L [240]. Trough concentrations exceeded the toxicity threshold of 2 mg/L in 57.7% (95% CI, 36.9 to 76.6) of those on 600 mg daily, and in 75% (95% CI, 19.4 to 99.4) of those who had undergone dose reduction to 300 mg daily. In a sensitivity analysis the proportions exceeding the toxicity threshold were similar when original trough concentration data were used: 67.7% (95% CI, 47.1 to 82.7) versus 60% (95% CI, 40.6 to 77.3) with imputed data at all doses.

Figure 2. Probability density distributions for efficacy target attainment of linezolid for subjects on 600 mg daily.

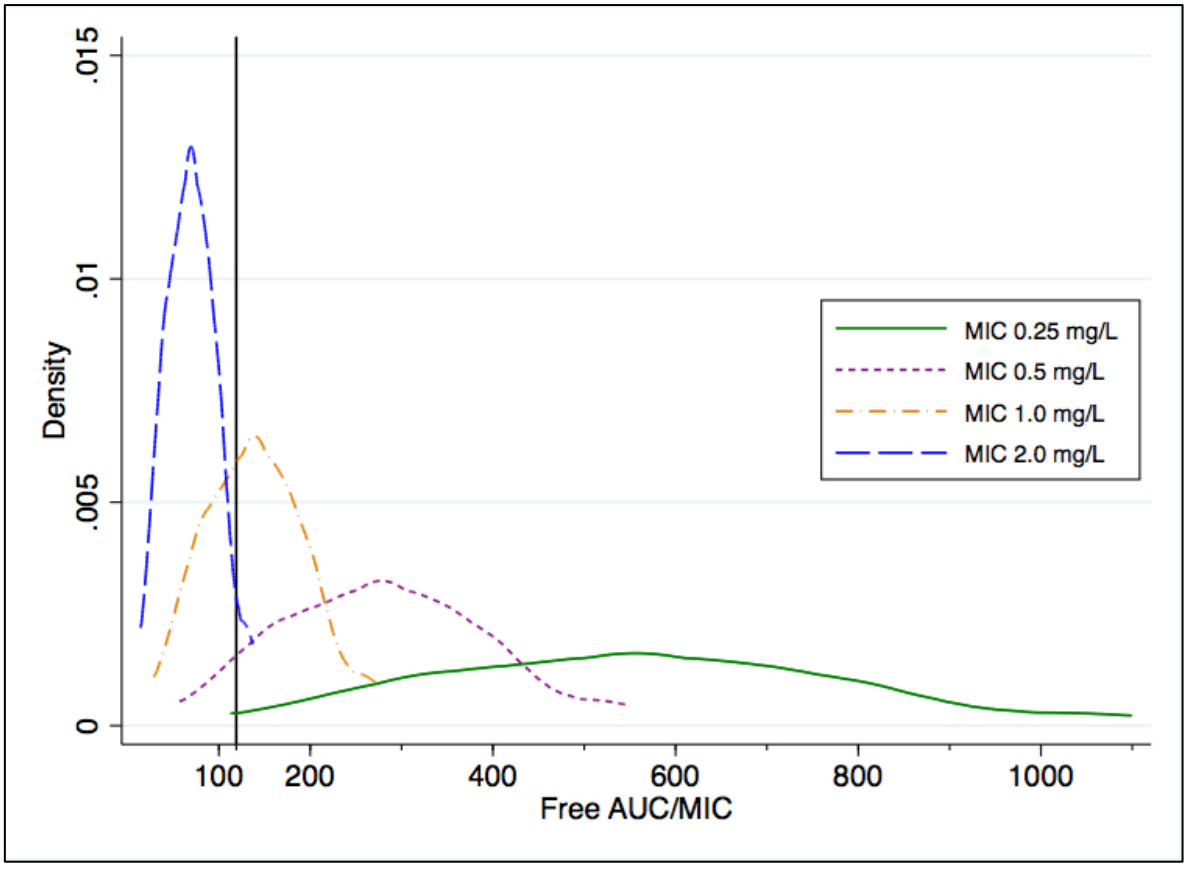


Figure 2. The solid vertical line on the x-axis represents the experimentally derived efficacy target *f*AUC/MIC$_{0\text{-}24}$ of 119. Note the log-scale on the x-axis.

Discussion

We characterized the PK of linezolid in 30 South African participants with drug-resistant TB and a high prevalence of HIV co-infection. We showed that age and weight were the most important predictors of linezolid exposure. A major finding was that the standard 600 mg dose resulted in exposures that reached efficacy targets, but a substantial

proportion of individuals were exposed to concentrations exceeding the known toxicity threshold. Of concern, at the critical concentration (1 mg/mL) efficacy targets would only be achieved in 61.5%, which has implications for the programmatic use of linezolid as resistance is expected to increase with more widespread use.

Despite its growing importance as a key drug for the treatment of drug-resistant TB, the optimal dose and duration of linezolid for this indication is unknown. There are very limited published PK data for linezolid in TB patients to help inform an effective dosing strategy that minimizes both mitochondrial toxicity and the emergence of resistance. Eight clinical studies reporting linezolid PK in TB treatment were identified in a recent systematic review [99] but these studies had four different dosing strategies and mostly did sparse sampling PK schedules, limiting their generalizability. Only two studies (n = 48) [63, 73] have evaluated linezolid PK at the standard dose for TB of 600 mg daily; all were HIV-negative and full PK profiles were only done in 10 participants [73]. Our study provides a comprehensive description of plasma linezolid concentrations at the recommended dose of 600 mg daily for drug-resistant TB and is the first to include HIV-positive patients.

We found high interindividual PK variability, as has been observed in patients with Gram-positive infections [94], particularly at early sampling time points, suggesting variable absorption delay. Most of the PK variability was unexplained by the covariates included in the regression model and was likely due to stochastic effects; however, this needs to be quantified with formal population PK modelling, possibly incorporating an absorption lag phase. Linezolid clearance was lower amongst participants who underwent dose reduction to 300 mg, which could be explained by channeling bias, as patients with lower linezolid clearance would have higher exposure and be more susceptible to toxicity, necessitating a dose reduction. Although the sample size was small, the median trough concentration with the reduced 300 mg daily doses exceeded the toxicity threshold of 2 mg/L in three of four participants. This finding emphasizes the need for toxicity monitoring with linezolid therapy, even after dose reduction for adverse events.

The median trough concentrations were higher in our cohort compared with the two previous studies of linezolid 600 mg daily in TB therapy [63, 73]. Although there is

substantial interstudy heterogeneity in linezolid PK parameters [99], our finding may suggest a longer terminal half-life with an attendant increased risk of toxicity in our population. A small clinical study found a trend towards an association between HIV infection and higher rates of linezolid toxicity [175]; if this association is confirmed in larger prospective cohorts, it is likely to be explained by predisposition to the high prevalence of neuropathy and limited bone marrow reserve in people with advanced HIV disease rather than higher linezolid exposure, which we did not find. We explored the potential PK drug-drug interaction between linezolid and lopinavir-ritonavir as an additional contributing factor to increased linezolid exposures and toxicity in HIV. An association between the use of lopinavir-ritonavir and linezolid trough concentrations was not detected in our cohort, but this needs confirmation with a larger sample size.

In a previous study, increasing age accounted for a small reduction (2%) in linezolid clearance in patients with Gram-positive infection [242], but did not contribute to the development of a population PK model of linezolid in TB [233], and did not influence linezolid exposures in a study of healthy volunteers [243]. By contrast, we showed a significant correlation between increasing age and linezolid trough concentrations, where every 10-year increment in age was associated with 43% higher trough concentrations; this finding needs to be validated in similar populations. We also found a significant association between weight and lower linezolid exposure in the multivariable model, an association previously reported [103]. These observations have implications for dose selection and could inform therapeutic drug monitoring (TDM) strategies for linezolid; for example, by targeting TDM to older patients and those with lower weights to prevent toxicity.

PK targets for efficacy have not been established for linezolid in TB treatment. Although C_{max}/MIC [211] and trough/MIC [105] have been associated with bacterial killing using *ex vivo* and *in vitro* models, the PK/PD index most consistently linked to linezolid activity in *M tuberculosis* is the *f*AUC_{0-24}/MIC ratio [72, 107, 244]. A hollow-fiber infection model, which recapitulates human drug exposure, showed that optimal mycobacterial kill was achieved at a *f*AUC_{0-24}/MIC ratio of 119 [107]; this was used as the PK/PD parameter in a recent simulation of published linezolid PK data to determine the probability of efficacy target attainment at wild type MIC values [99]. Using data from 10 patients with full PK profiles, with an estimated median AUC_{0-24} of 98.6 mg.h/L [233], those

simulations predicted that 45% would fail to achieve the target at a daily dose of 600 mg. Reassuringly, in our participants linezolid exposures were higher (median AUC_{0-24} 200.2 mg.h/L), translating into probability of target attainment of 100% across the MIC distribution in baseline isolates and 96% at the population wild type MIC cut-off of 0.5 mg/kg, supporting the efficacy of the 600 mg daily dose. However, linezolid exposures did not exceed the putative efficacy threshold at the critical concentration of 1 mg/L in 38% of our subjects. With the expanding use of linezolid for TB treatment it will be essential to monitor for evidence of 'MIC creep' in the population.

Unlike the PK/PD parameter for efficacy, the linezolid toxicity threshold is relatively well-defined as a trough concentration of 2 mg/L, supported by clinical evidence [83] as well as data from pre-clinical models showing that mitochondrial toxicity is related to trough concentrations [105]. Although a 600 mg daily dose was likely to reach the efficacy target in our cohort, almost 58% also exceeded this threshold concentration for linezolid toxicity, clearly illustrating the narrow therapeutic window of linezolid. In murine models, linezolid's sterilizing ability is dose-related and can occur within 2 months of effective combination therapy [172, 173]. In TB patients, neurological toxicity tends occur late, usually after 2 months of therapy [110]. Based on these observations, an appealing dosing strategy could be to provide higher linezolid doses (1,200 mg daily) for an initial 'intensive phase' of treatment, followed by either discontinuation, dose reduction, or intermittent dosing [149] that allows longer periods within the PK safety window. This strategy needs to be evaluated in prospective studies.

We acknowledge a number of limitations of our study, including the inability of non-compartmental analysis to assess intra-individual PK variability, evaluation at only a single time point during treatment, an incomplete PK profile and non-steady state dosing for one participant each, and small numbers of participants receiving the reduced 300 mg dose. Importantly, we had to impute the trough concentrations for six participants due to extremely high values after suspected unobserved dosing prior to the 24-hour sample. If anything, inclusion of the original data would have biased the results towards higher trough concentrations and overall exposures. Thus, our reported findings may represent a conservative estimate of both efficacy and toxicity target attainment.

In conclusion, we found substantial variability in linezolid drug concentrations in this cohort of patients with drug-resistant TB and a high prevalence of HIV infection. Much of this variability was unexplained, but age and weight were identified as predictors of trough concentrations and exposure, respectively. The standard 600 mg dose is likely to achieve efficacy targets for *M tuberculosis* isolates with linezolid wild type MICs. The clinical impact of this needs to be evaluated by linking linezolid PK to toxicity and efficacy endpoints. In the meantime, the expanding use of linezolid 600 mg daily for drug-resistant TB should be supported by programmatic surveillance of MICs and adverse events. Alternative dosing strategies and TDM should be explored to optimize the use of this important but toxic antituberculosis agent.

CHAPTER 4

Linezolid toxicity in patients with drug-resistant tuberculosis: a prospective cohort study

ABSTRACT

Background: Linezolid is recommended for treating drug-resistant tuberculosis. Adverse events are a concern to prescribers but have not been systematically studied at the standard dose, and the relationship between linezolid exposure and clinical toxicity is not completely elucidated.

Patients and Methods: We conducted an observational cohort study to describe the incidence and determinants of linezolid toxicity, and to determine a drug exposure threshold for toxicity, among patients with rifampicin-resistant tuberculosis in South Africa. Linezolid exposures were estimated from a population pharmacokinetic model. Mixed-effects modelling was used to analyse toxicity outcomes.

Results: 151 participants, 63% HIV-positive, were enrolled and followed for a median of 86 weeks. Linezolid was permanently discontinued for toxicity in 32 (21%) participants. Grade 3 or 4 linezolid-associated adverse events occurred in 21 (14%) participants. Mean haemoglobin concentrations increased with time on treatment (0.03 g/dL per week; 95% CI, 0.02 to 0.03). Linezolid trough concentration, male sex, and age (but not HIV-positivity) were independently associated with a decrease in haemoglobin > 2 g/dL. Trough linezolid concentration of 2.5 mg/L or higher resulted in optimal model performance to describe changing haemoglobin and treatment-emergent anaemia (adjusted odds ratio 2.9; 95% CI, 1.3 to 6.8). Single nucleotide polymorphisms 2706A>G and 3010G>A in mitochondrial DNA were not associated with linezolid toxicity.

Conclusions: Permanent discontinuation of linezolid was common, but linezolid-containing therapy was associated with average improvement in toxicity measures. HIV co-infection was not independently associated with linezolid toxicity. Linezolid trough concentration of 2.5 mg/L should be evaluated as a target for therapeutic drug monitoring.

Introduction

Rifampicin-resistant tuberculosis (RR-TB) accounts for an expanding proportion of incident global TB cases and is an ongoing threat to EndTB targets. [29] Linezolid is a repurposed oxazolidinone antimicrobial with bactericidal activity against *M. tuberculosis* [74]. Inclusion of linezolid in treatment regimens for RR-TB is associated with treatment success and mortality reduction [38, 63]; as a result, WHO guidelines now recommend linezolid as a preferred agent for RR-TB [61].

The major drawback of linezolid is binding to human mitochondrial 16S ribosomal RNA (rRNA), which has a homologous structure to the *M. tuberculosis* target site [77], resulting in dose-related mitochondrial toxicity that manifests most commonly as bone marrow suppression and peripheral neuropathy. These toxic effects may be treatment-limiting [41, 81]. The incidence and risk factors for linezolid toxicity have not been systematically studied in TB programs [245], particularly in populations from sub-Saharan Africa with high rates of HIV co-infection, which could increase the risk of toxicity [175]. Factors possibly associated with linezolid toxicity include age, sex, and polymorphisms in mitochondrial DNA (mtDNA) [82, 109, 246]. Estimating frequency and identifying risk profiles for serious linezolid toxicity will support deployment of this drug in programmatic settings.

An approach to mitigate toxicity is through optimized linezolid dosing, which requires characterization of the exposure-toxicity relationship [238]. Standard linezolid dosing in RR-TB (600 mg daily) is likely to achieve an *in vitro* efficacy target for *M tuberculosis* and reduce the emergence of resistance [95]. Trough concentrations are inversely correlated with mitochondrial function, [83] haemoglobin concentration in mouse models, [247, 248] and clinical toxicity among patients with Gram-positive infection. [108, 109] Pharmacokinetic (PK)-toxicity targets have been suggested from small clinical studies, but these are not adequately established for patients with TB. [83, 109] Linezolid trough concentrations correlate with area under the concentration-time curve (AUC, the target PK parameter for efficacy) [249], suggesting a potential role for therapeutic drug monitoring (TDM) if a concentration threshold target for clinical toxicity is defined [250].

We aimed to describe the incidence and determinants of linezolid toxicity, and to determine a drug exposure threshold for toxicity, among patients with RR-TB in a programmatic setting with a high HIV burden.

Patients and methods

Design and population

This analysis was nested in a prospective observational cohort study (PROBeX) conducted at 3 drug-resistant TB referral hospitals in South Africa. The parent PROBeX study recruited 195 adults with known HIV status and culture-confirmed RR-TB who were initiating treatment with a bedaquiline-containing regimen between April 2016 and March 2018 [128]. During the study period local treatment guidelines recommended an 18- to 24-month regimen. Linezolid was provided at a dose of 600 mg daily, with reduction to 300 mg daily at the discretion of treating clinicians if toxicity developed. Linezolid was recommended for the full treatment course if tolerated, but the duration was determined by treating clinicians. Treatment decisions were informed by clinical assessments and routine toxicity screening which included monthly full blood counts; linezolid TDM was not performed.

Procedures

Participants were followed until 6 months after completion of therapy, or up to 24 months after study entry, at the start of bedaquiline therapy. Study visits occurred monthly during the first 6 months of therapy, then 6-monthly until study exit. Phlebotomy was performed at every visit for full blood count and lactate; results of these tests performed in routine care outside of study visits were also obtained. The modified Brief Peripheral Neuropathy Scale (BPNS) was used to screen for peripheral neuropathy. [129] We assessed visual acuity using logMAR charts and colour vision using 14-plate Ishihara charts to screen for optic neuropathy. Neuropathy screening was done at every study visit. Mitochondrial DNA (mtDNA) was extracted from stored whole blood and the 16S rRNA gene sequenced to detect two SNPs (2706A>G and 3010G>A) previously associated with linezolid-induced mitochondrial toxicity [82].

PK data

We did intensive PK sampling (pre- and at 1, 2, 3, 4, 5, 6, and 24 hours post-dose) on a subgroup of 21 participants at month 2 and sparse (pre-dose) PK sampling for the full

cohort at months 1, 2, and 6 after initiation of linezolid therapy. Linezolid concentrations were measured in the Division of Clinical Pharmacology at the University of Cape Town using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay [249]. We developed a population PK model using these data and derived average linezolid area under the concentration-time curve over 24 hours (AUC_{0-24}) and trough values for individual participants, based on body weight and time-varying linezolid dose [251].

Outcome definitions

The main outcome was linezolid toxicity measured by cytopenia, peripheral and optic neuropathy, and hyperlactatemia. We defined anaemia, thrombocytopenia, leukopenia, and hyperlactatemia according to Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 2.1. Peripheral neuropathy was graded according to the modified BPNS score. [252] Optic neuropathy was defined as an increase of 0.3 on the logMAR score in either eye [253] or a reduction in colour vision score of > 2 [254]. We performed exploratory data analysis to identify thresholds for toxicity measures by observing distribution and trends of haematological parameters and lactate over time and relationship with baseline values. Early discontinuation of linezolid was defined as a permanent stop prior to 6 months of therapy.

Analysis

Kaplan-Meier survival curves were computed to analyse and plot the timing of event onset; median times were reported for participants who experienced events. The primary outcome of interest was change in haemoglobin concentration from baseline. The key covariates were linezolid exposure, duration on linezolid, age, sex, and HIV status. Effect of risk-associated mtDNA SNPs was also explored. To describe changing continuous outcomes, we fitted linear mixed-effects regression models incorporating baseline controlling variables and time-varying covariates (linear time effect and linezolid exposure). We used conditional logistic regression for repeated toxicity events and computed marginal probabilities to represent risk; this approach was selected to incorporate multiple recuring events and account for within-individual correlation through inclusion of participant-specific random effects [255]. Internal model validation was performed using a k-fold cross-validation procedure [256]. We performed a piecewise (broken stick) regression procedure to identify the optimal threshold value of

linezolid exposure that predicted clinical toxicity based on best model fit of linear regression models as measured by Akaike Information Criteria at multiple values of linezolid trough concentrations [257, 258].

The study was not formally powered as the predictors of linezolid toxicity or PK-pharmacodynamic relationships are not well characterized. A *post hoc* power calculation showed that a sample size of around 150 participants would have > 95% probability of detecting a 95% confidence interval with precision (width) of at least 0.18 for anaemia, given a standard deviation of 0.51 in our sample.

All analyses were performed with Stata/BE 17.0 (Statacorp).

Ethics

This study was approved by the Human Research Ethics Committee at the University of Cape Town (437/2016), Albert Einstein College of Medicine, and Emory University. All participants provided written informed consent prior to performance of study procedures. The study was conducted and reported according to STROBE guidelines.

Results

Characteristics of study population

We included 151 participants out of 195 enrolled in the parent cohort; 44 were excluded because of no documented linezolid prescription (n = 38) or absent toxicity measure after starting linezolid (n = 6). Baseline characteristics are shown in (Table 1); 63% were HIV-positive, and 66% had fluoroquinolone-resistant TB. In addition to linezolid, all participants received bedaquiline; clofazimine, levofloxacin, pyrazinamide, terizidone, and para-aminosalicylic acid were provided to over 95%; and ethambutol was prescribed for 74 participants (49%). Prior to starting linezolid, the median haemoglobin was 11.8 g/dL (range 6.4 – 17.9). Median follow up from start of linezolid therapy was 86 weeks (range 3 - 183). A single A>G substitution at position 2706 was detected in 124 (87%) participants; no SNPs were detected at position 3010.

Table 1. Baseline characteristics.

	n (%) or median (IQR)	Denominator
Age, yrs	34 (28-42)	151

Female sex	84 (56%)	151
Ethnicity		151
Black	127 (84%)	
Mixed race	22 (15%)	
Other	2 (1%)	
Weight, kg	53 (47-60)	150
BMI, kg·m^{-2}	20 (18-22)	149
HIV-positive	95 (63%)	151
CD4 count, cells/mm^3	212 (111-438)	91
Antiretroviral therapy	81 (85%)	95
Previously treated TB	111 (74%)	151
Resistance pattern of baseline isolate		141
MDR	12 (9%)	
Pre-XDR (injectable)	36 (26%)	
Pre-XDR (fluoroquinolone)	32 (23%)	
XDR	61 (43%)	
Creatinine, µmol/L	62 (51-71)	150
Creatinine clearance, mL/min	105 (88-125)	149
Haemoglobin, g/dL	11.8 (10.4 – 13.2) Range: 6.4 – 17.9	148
White blood cell count (x 10^9 cells/L)	7.3 (5.3 – 9.5) Range: 1.4 – 27.1	148
Platelets (x 10^9/L)	345 (271 - 489) Range: 132 - 1131	148
Venous lactate, mmol/L	1.7 (1.3 – 2.3) Range: 0.8 – 6.5	115
BPNS grade		121
0	100 (83%)	
1	17 (14%)	
2	4 (3%)	
LogMAR score (right)	0 (range 0 - 1.0)	113
LogMAR score (left)	0 (range 0 - 0.8)	111
Ishihara score	6 (6-6)	123

mtDNA 16S rRNA polymorphism		
2706A>G	124 (87%)	142
3010G>A	0 (0)	142

Table 1. BMI, body mass index; MDR, multi-drug resistant (resistance to rifampicin plus isoniazid); XDR, extensively drug resistant (additional resistance to fluoroquinolones and injectable agents); pre-XDR (additional resistance to either fluoroquinolones or injectable agents); BPNS, modified brief peripheral neuropathy score.

Linezolid therapy and PK

The starting linezolid dose was 600 mg daily in 148 participants and 300 mg in three. The median duration of linezolid therapy, excluding treatment interruptions, was 336 days (IQR 159 – 506; range 6 - 862). Linezolid dose was reduced for 31 (21%) participants at a median time of 69 days (IQR 36 – 147). Linezolid was permanently discontinued in 32 (21%) participants at a median time of 60 days (IQR 20 - 99); 10 (31%) patients had either dose reduction or interruption prior to early discontinuation (Table 2 and Figure 1).

Table 2. Details of linezolid interruption.

Parameter	**n = 151**
Linezolid changes	
- Dose reduction	31 (21%)
- Interruption then dose reduction	6 (4%)
- Interruption then same dose	10 (7%)
- Early discontinuation	32 (21%)
Linezolid duration	
- Until first interruption/change	69 days (IQR 36 – 147; range 4 – 530)
- Duration of interruption	42 days (IQR 28 – 85; range 5 – 315)
- Until early discontinuation	60 days (IQR 20 – 99; range 12 – 179)
- Total duration	336 days (IQR 159 – 506; range 6 - 862)

Table 2. Data are number (percent) or median (interquartile range). Early discontinuation defined as permanent discontinuation before 6 months. 10 (31%) patients had either dose reduction or interruption prior to early discontinuation. Total duration excludes time off linezolid during treatment interruptions.

Figure 1. Time to early discontinuation of linezolid at 52 weeks.

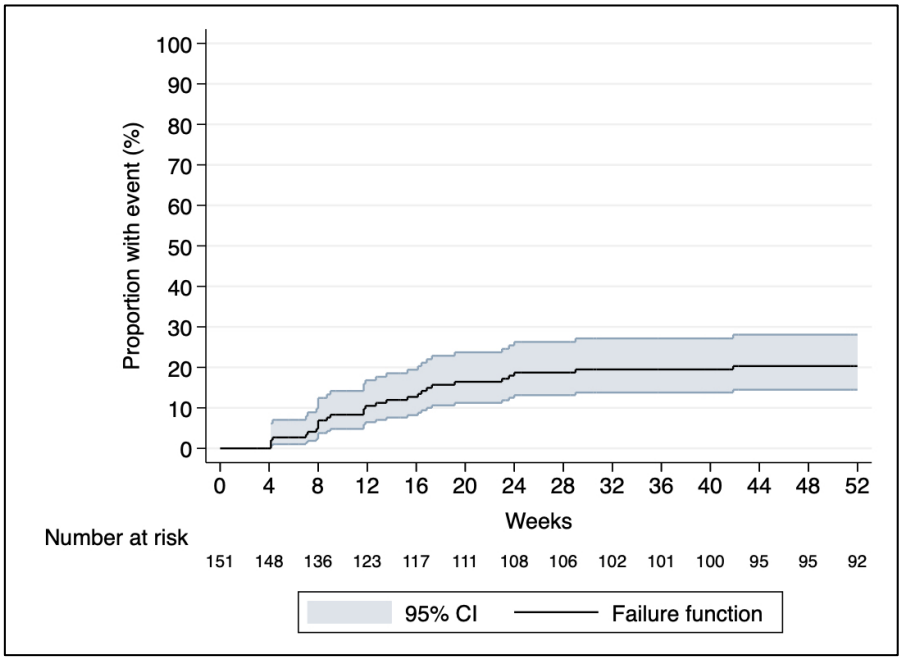


Figure 1. Kaplan-Meier plot with survival estimates for early discontinuation of linezolid, defined as permanent stop prior to completing 6 months of therapy.

The individual PK parameters were derived from a population PK model based on observed concentrations for 95 participants and were predicted (based on weight and dose) for the other 56 participants with no measured linezolid concentrations. Median linezolid $AUC_{0\text{-}24}$ was 168.9 mg·h/L (IQR 143 – 194) and trough concentration was 2.1 mg/L (1.8 – 2.3) for the 600 mg dose (Figure 2). There was an exponential relationship between AUC and trough concentrations, which were highly correlated (Figure 3).

Figure 2. Linezolid secondary pharmacokinetic parameters, by dose.

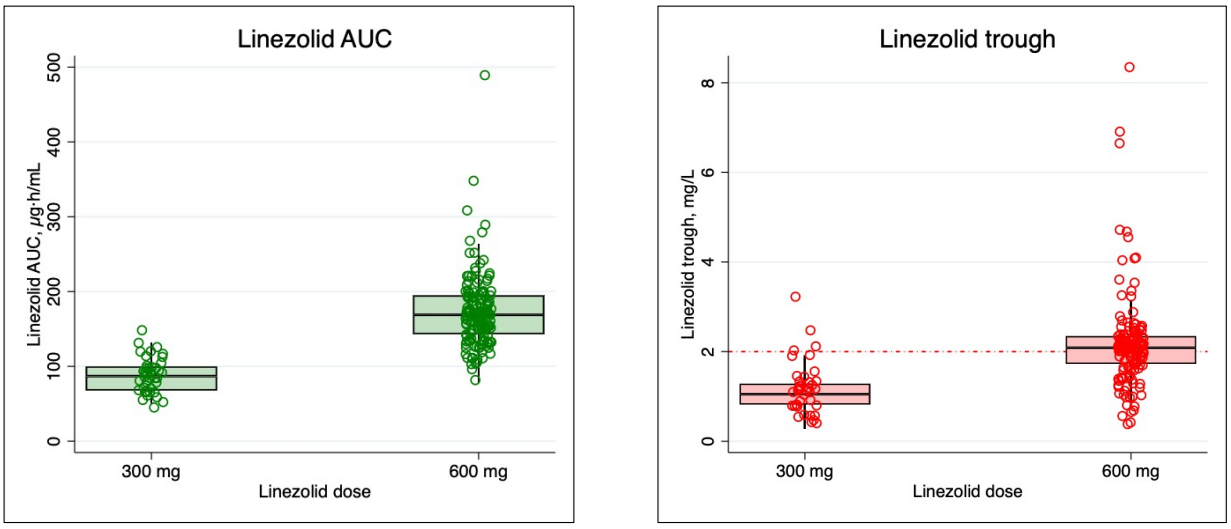


Figure 2. Distribution of linezolid area under the concentration-time curve (AUC), left panel, and trough

concentrations, right panel, by dose. Estimates derived from population pharmacokinetic model. Open circles are individual values for each study visit, boxes indicate median and interquartile ranges, whiskers indicate upper adjacent value (1.5x IQR). Dashed red line indicates trough value of 2 mg/L, the putative toxicity threshold derived elsewhere.

Figure 3. Correlation between linezolid AUC and trough concentrations.

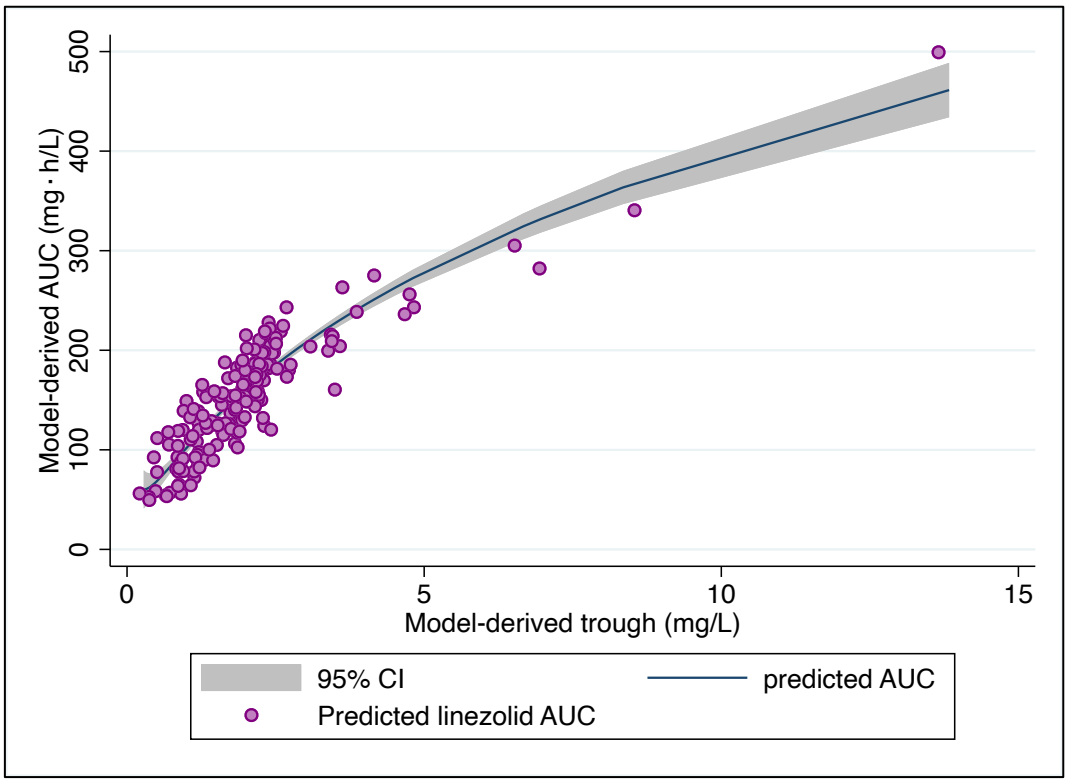


Figure 3. Secondary pharmacokinetic parameters derived from population pharmacokinetic model. AUC, area under the concentration-time curve; 95% CI, confidence interval.

Linezolid toxicity events

Cumulative incidence of any new grade anaemia or peripheral neuropathy DAIDS event at 6 months was 39% (95% CI, 31 – 47) and 20% (95% CI, 14 – 27), respectively, with similar median time to experiencing the event: 11 weeks (IQR 7 - 17) for anaemia and 10 weeks (IQR 7 - 23) for neuropathy (Figure 4). New grade 3 or 4 events occurred in 21 participants: cumulative incidence 14% (95% CI, 9 - 21) at 6 months. 16 participants had reductions in visual acuity with a cumulative incidence of 12% (95% CI, 8 – 20) at 24 months; median time to onset was 10 weeks (range 5 - 79 weeks). Linezolid was dose-reduced or permanently discontinued in 5 participants with reduced visual acuity. Only one participant experienced reduction in colour vision (Table 3).

Figure 4. Time to development of any grade adverse event for anaemia or peripheral neuropathy.

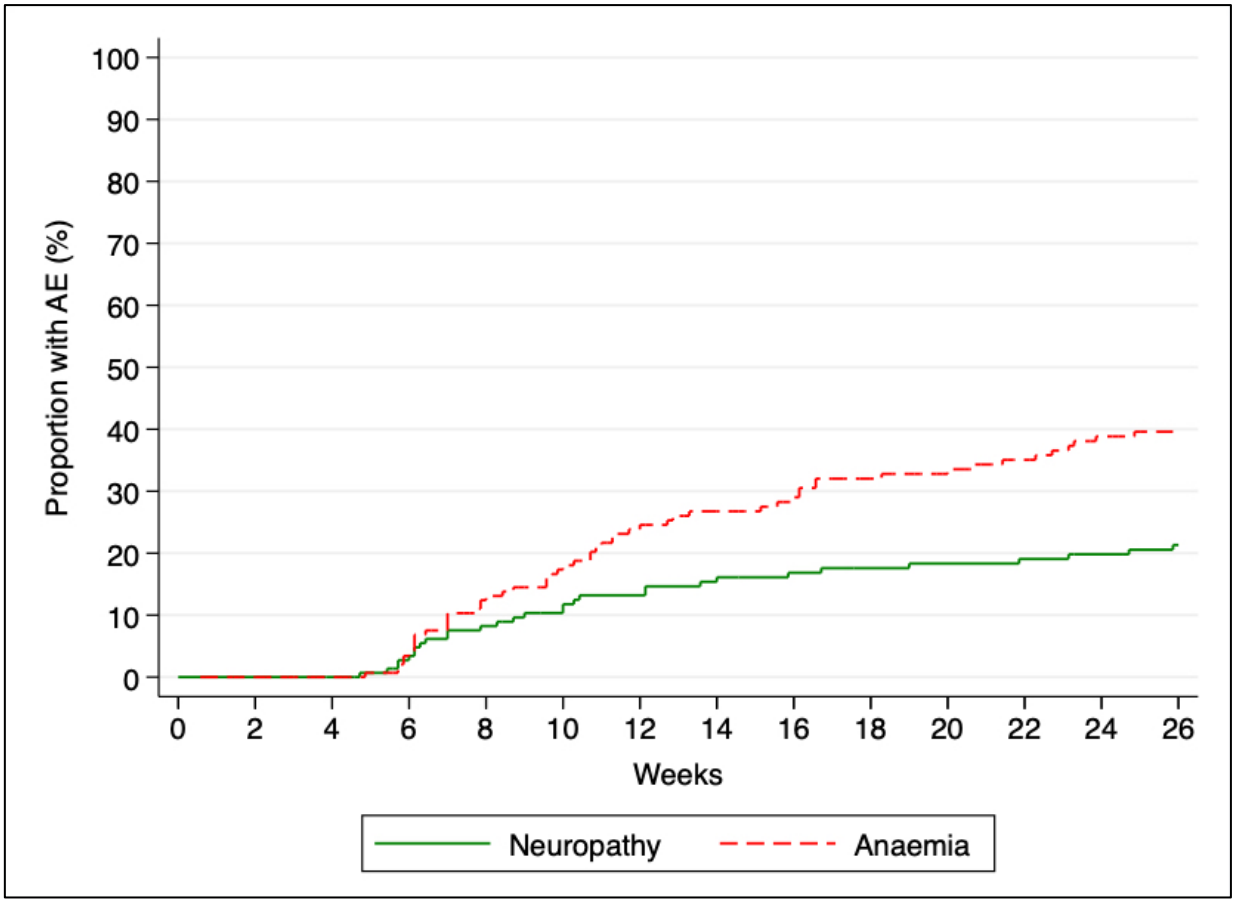


Figure 4. Kaplan-Meier plot with superimposed individual survival estimates for anaemia and neuropathy. Anaemia defined as any new grade on DAIDS grading and neuropathy defined as any new grade on BPNS score. AE, adverse event.

Table 3. New adverse events after starting linezolid.

	Number of participants with any event (n = 151)	Grade 1	Grade 2	Grade 3	Grade 4	Cumulative incidence of any grade at 6 months (95% CI)	Event rate (per 100 person-weeks) (95% CI)
Anaemia	58	25 (17)	14 (9)	13 (9)	6 (4)	39% (31 – 47)	0.8 (0.6 – 1.0)
Thrombocytopenia	10	8 (5)	2 (1)	0	0	6% (3 – 11)	0.1 (0.0 – 0.2)
Leukopenia	6	5 (3)	0	1 (0.6)	0	4% (2 – 9)	0.1 (0.0 – 0.2)
Peripheral neuropathy	37[a]	32 (26)	4 (3)	0	1 (0.8)	20% (14 – 27)	0.4 (0.3 – 0.5)
Reduced visual acuity	16[b]	-	-	-	-	9% (5 – 15)	0.1 (0.1 – 0.2)
Worsening colour vision	1[c]	-	-	-	-	-	-
Hyperlactatemia	51[d]	43 (37)	8 (7)	-	-	31% (24 – 40)	0.6 (0.5 – 0.8)

[a] n = 121, [b] n = 119, [c] n = 123, [d] n = 115

Table 3. These data are for the highest-grade adverse event experienced by individual participants; percentages with event from total number of participants in parentheses. Anaemia, thrombocytopenia, leukopenia, and hyperlactatemia were defined according to Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 2.1. Data on grade 3 or 4 hyperlactatemia was not collected as associated symptoms were not ascertained and pH was not measured. Peripheral neuropathy was graded according to the modified BPNS score. Optic neuropathy was defined as an increase of 0.3 on the logMAR score in either eye or a reduction in color vision score of > 2 on a 14-plate Ishihara chart.

Additional toxicity outcomes were defined based on the observed data: anaemia, haemoglobin reduction > 2 g/dL; thrombocytopenia, platelet reduction > 250 x 10^9/L; leukopenia, white cell count reduction > 4 cells x 10^9/L; and hyperlactatemia, lactate increase > 1.5 mmol/L (Table 4). Using these definitions, cumulative incidence of anaemia at 6 months was 33% (95% CI, 26 - 41), thrombocytopenia 16% (95% CI, 11 - 23), leukopenia 20% (95% CI, 14 - 28), and hyperlactatemia 15% (95% CI, 10 - 22).

Table 4. Toxicity outcomes defined from observed data.

Toxicity outcome	Measure	Absolute change	Percentile	Percentage change	Percentile
Anaemia	**Haemoglobin, g/dL**	**- 2**	**25**	**-20**	**10**
Thrombocytopenia	**Platelet count, x10^9/L**	**-250**	**10**	**-50**	**10**
Leukopenia	**White cell count, x10^9/L**	**-4**	**10**	**-50**	**10**
Hyperlactataemia	**Lactate, mmol/L**	**+1.5**	**5**	**+25**	**10**

Relationship between linezolid exposure and toxicity

A linezolid trough concentration of 2.5 mg/L resulted in optimal model fit to describe association with change in haemoglobin compared to other breakpoint values using piecewise regression (Table 5 and Figure 5).

Table 5. Akaike Information Criteria (AIC) estimates for range of linezolid trough spline terms in mixed-effects linear regression model for change in haemoglobin (Hb).

Model (trough cut off)	AIC
No spline term	3805.219
1.0 mg/L	3801.814
1.1 mg/L	3801.091
1.2 mg/L	3800.48
1.3 mg/L	3800.344
1.4 mg/L	3800.467
1.5 mg/L	3800.643

1.6 mg/L	3800.698
1.7 mg/L	3800.362
1.8 mg/L	3800.5
1.9 mg/L	3800.615
2.0 mg/L	3800.682
2.1 mg/L	3800.333
2.2 mg/L	3800.411
2.3 mg/L	3800.11
2.4 mg/L	3799.834
2.5 mg/L	3799.797
2.6 mg/L	3800.131
2.7 mg/L	3800.434
2.8 mg/L	3800.706
2.9 mg/L	3800.949
3.0 mg/L	3801.048

Table 5. Trough value 2.5 mg/L had the lowest AIC and discriminated best for observed events (Fig. 3).

Figure 5. Change in haemoglobin from baseline values by linezolid trough concentration.

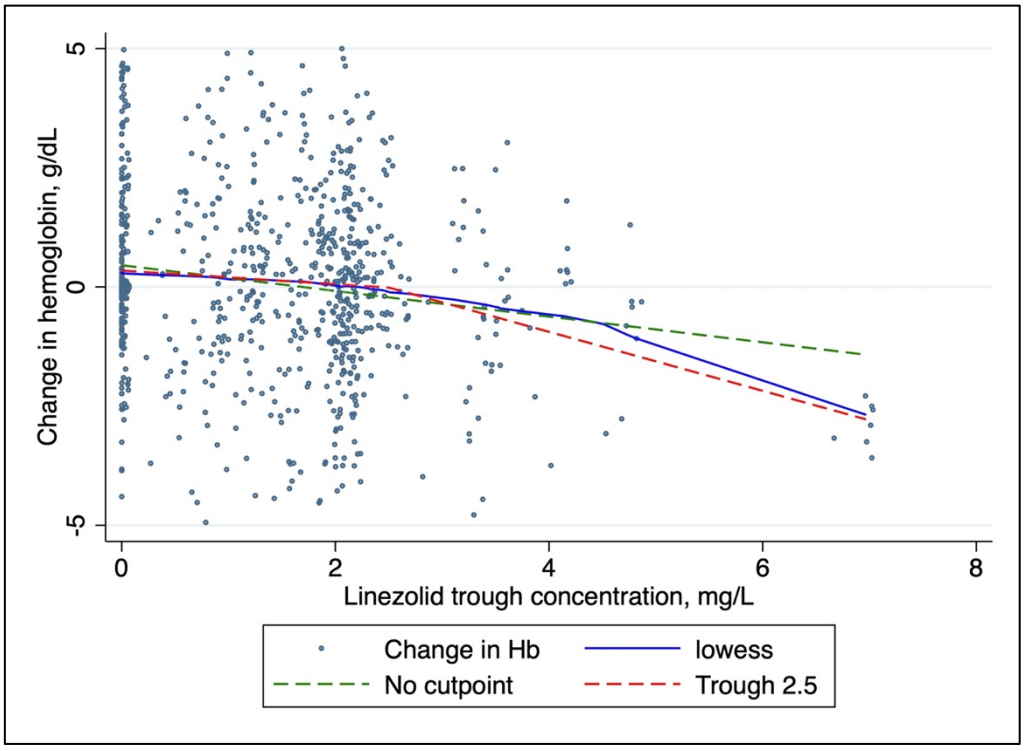


Figure 5. Mixed-effects linear regression model fit for change in haemoglobin with spline term for trough concentration at 2.5 mg/L, demonstrating model fit to the data. Blue dots indicate observed values, solid line indicates locally weighted smoothing, dashed lines indicate model-predictions.

There was a clear time trend for the onset of anaemia, defined as a drop in haemoglobin > 2 g/dL, during the first 6 months of linezolid therapy: of the 47 participants who experienced anaemia, 43 (91%) events occurred within 120 days. 8 out of 9 (89%) participants with a linezolid trough concentration above 2.5 mg/L in this period had anaemia; 38% (21/55) with trough concentrations below this threshold had no anaemia (Figure 6).

Figure 6. Observed relationship between anaemia events and linezolid trough concentrations during the first 6 months of linezolid therapy.

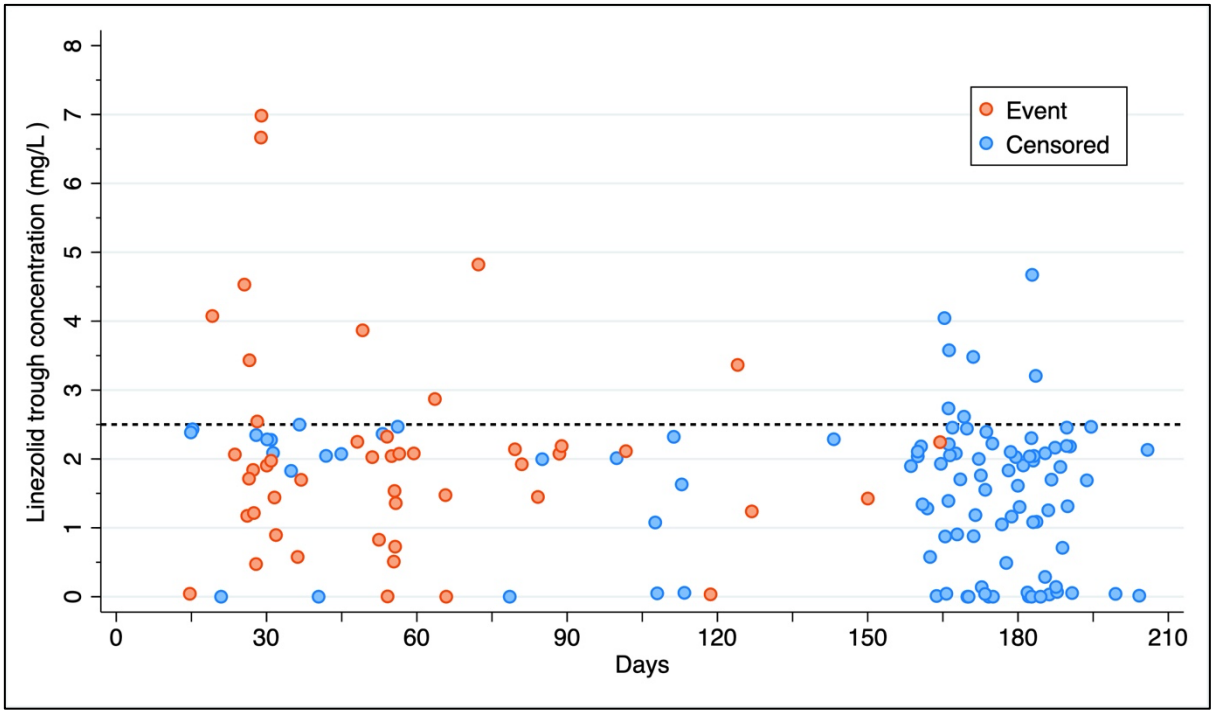


Figure 6. Events defined as reduction in haemoglobin > 2 g/dL in red circles; censoring at 6 months without anaemia, and for lost to follow up, and death in blue circles. Dashed line indicates trough concentration of 2.5 mg/L.

For trough concentrations below 2.5 mg/L, the median fAUC was 113 (IQR 98 - 128) and 40% of fAUC estimates were above the putative efficacy threshold of 119 with linezolid dosing at 600 mg (Figure 7).

Figure 7. Linezolid *f*AUC if trough concentrations < 2.5 mg/L.

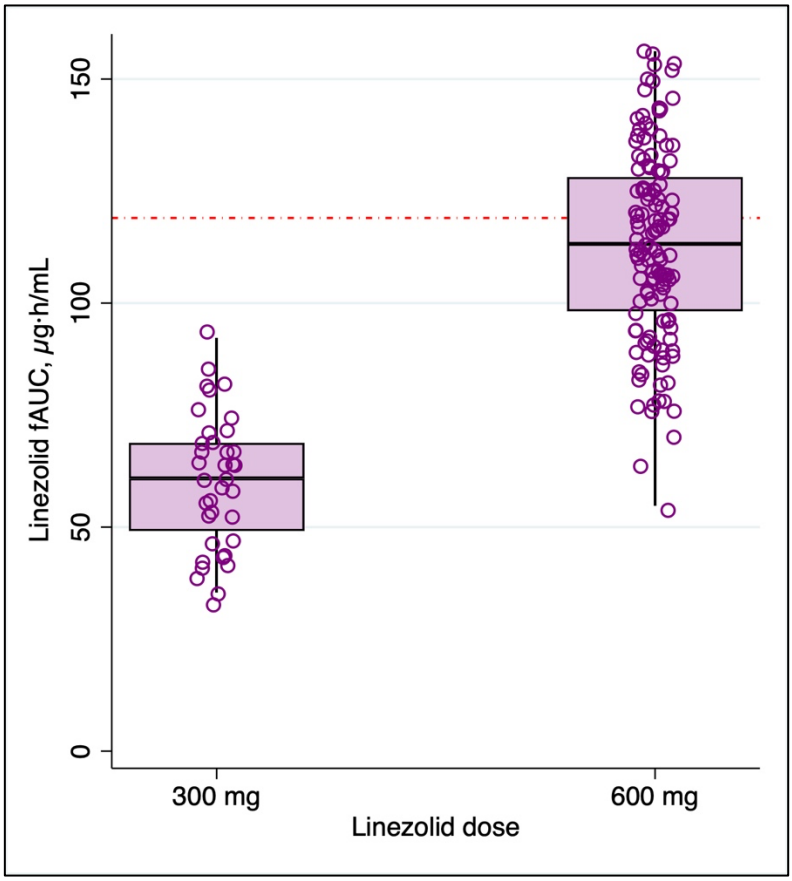


Figure 7. Dashed red line indicates efficacy target of *f*AUC/MIC 119. Open circles indicate observed values for *f*AUC.

Factors associated with linezolid toxicity measures

Platelet counts decreased over time on average; there was a negative correlation with baseline values (rho -0.65, $p < 0.001$) and increasing hemoglobin (Rho -0.26, $p < 0.001$), suggesting platelet reductions were related to positive treatment effect (Figures 8 and 9).

Figure 8. Relationship between change in haemoglobin and change in platelet count.

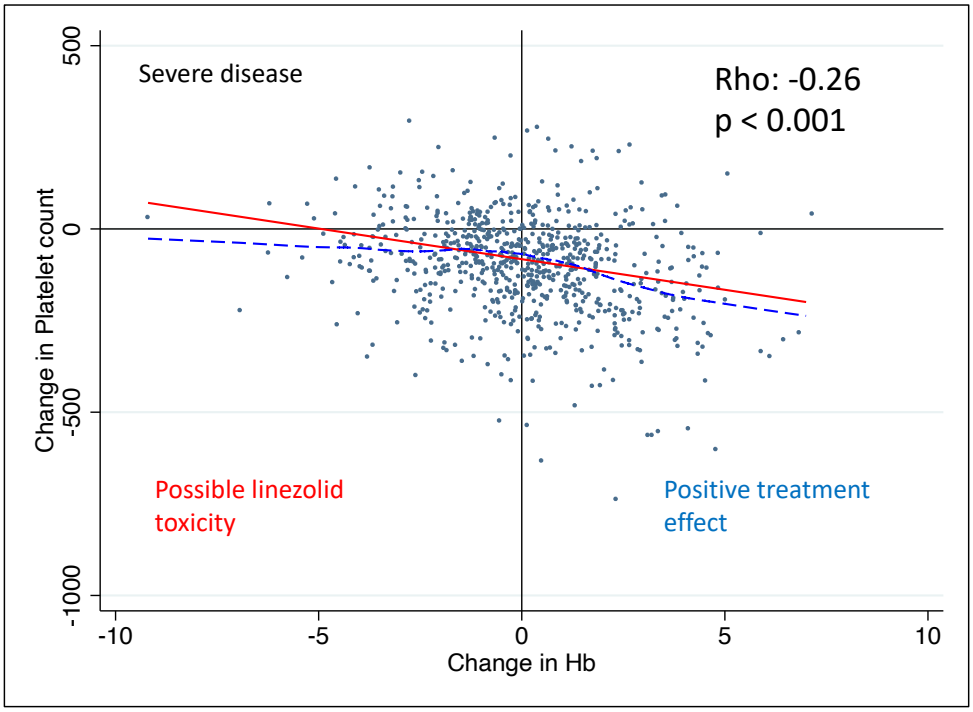


Figure 8. Dots indicate observed values, red solid line is fitted regression line, blue dashed line is locally weighted smoother. Data show that platelet count goes down as haemoglobin increases, suggesting positive treatment effect.

Figure 9. Relationship between change in platelet count and baseline platelet count.

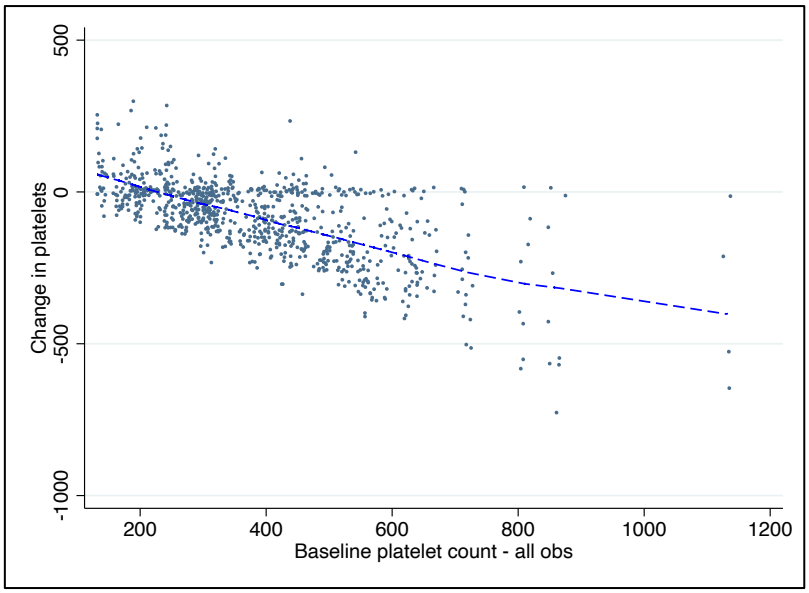


Figure 9. Dots indicate observed values, blue dashed line is locally weighted smoother. Demonstrates greater reduction in platelet counts with higher baseline values (rho -0.65, p < 0.001).

Mean haemoglobin was predicted to increase with time on treatment (0.03 g/dL per week; 95% CI, 0.02 to 0.03) and with a higher pre-treatment haemoglobin (0.6 g/dL;

95% CI, 0.5 to 0.7); and to decrease with increasing linezolid trough concentrations (-0.2 g/dL per 1 mg/L; 95% CI, -0.3 to -0.1), HIV-positivity (-0.5 g/dL; 95% CI, -1.0 to -0.1), and age (-0.3 g/dL per 10 years; 95% CI, -0.5 to -0.1) (Figures 10 and 11A). 31% of total variability was due to inter-individual variability. Model-predicted haemoglobin at 4 weeks was 8.2 g/dL (95% CI, 7.8 to 8.8) for an HIV-positive participant with the lowest pre-treatment haemoglobin of 6.4 g/dL (at observed values of other parameters).

Figure 10. Predictors of longitudinal haemoglobin measures over the study period.

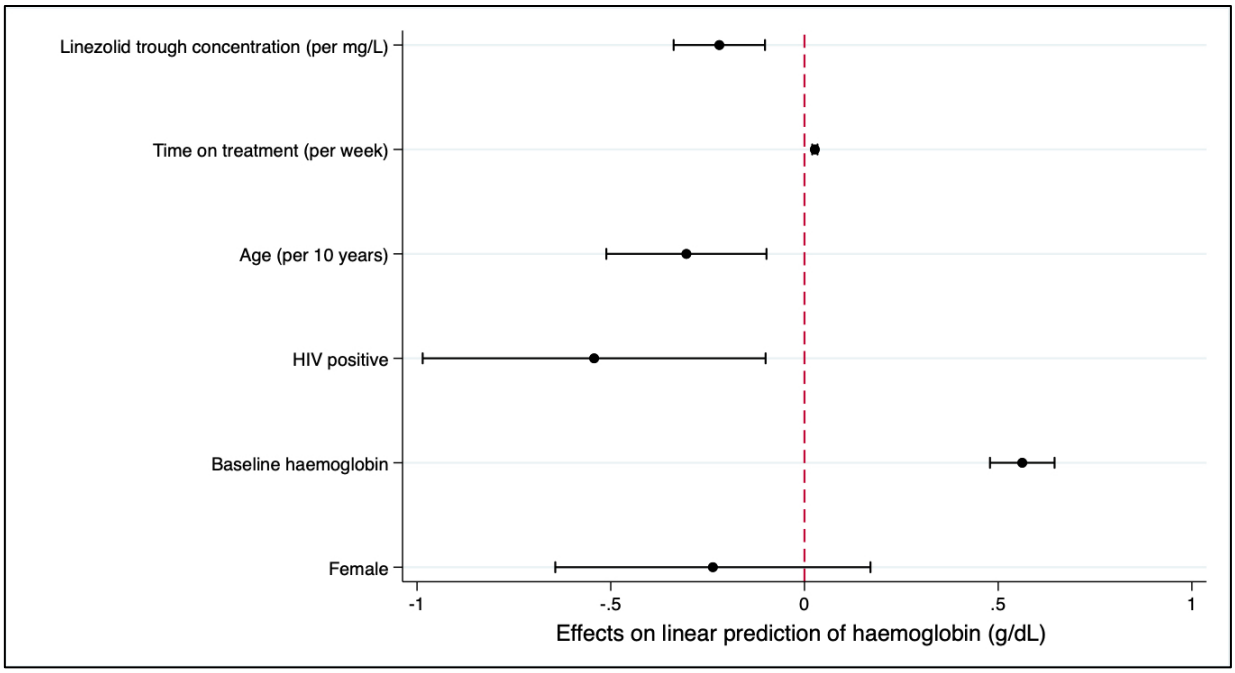


Figure 10. Estimates of mean effects on haemoglobin from the mixed-effects linear regression model. Dots indicate point estimate; black lines indicate 95% confidence interval; dashed red line indicates no effect.

Average platelet count, white blood cell count, and lactate decreased over time when adjusted for baseline values, HIV status, age, gender, and linezolid trough concentrations (supplement S4B-D). Lactate increase was associated with linezolid trough concentrations (0.08 mmol/L increase per 1 mg/L linezolid trough, 95% CI 0.01 to 0.2). There was an inverse association between linezolid trough concentrations and both platelet count (-11.4 × 10^9/L, 95% CI -19.7 to -3.1) and white cell count (-0.2 cells × 10^9/L, 95% CI -0.3 to -0.02). Sensitivity analysis was done for all outcomes including only PK estimates from measured concentrations, without substantial change in parameter estimates.

Figures 10 (A - D). Change in toxicity measures over time.

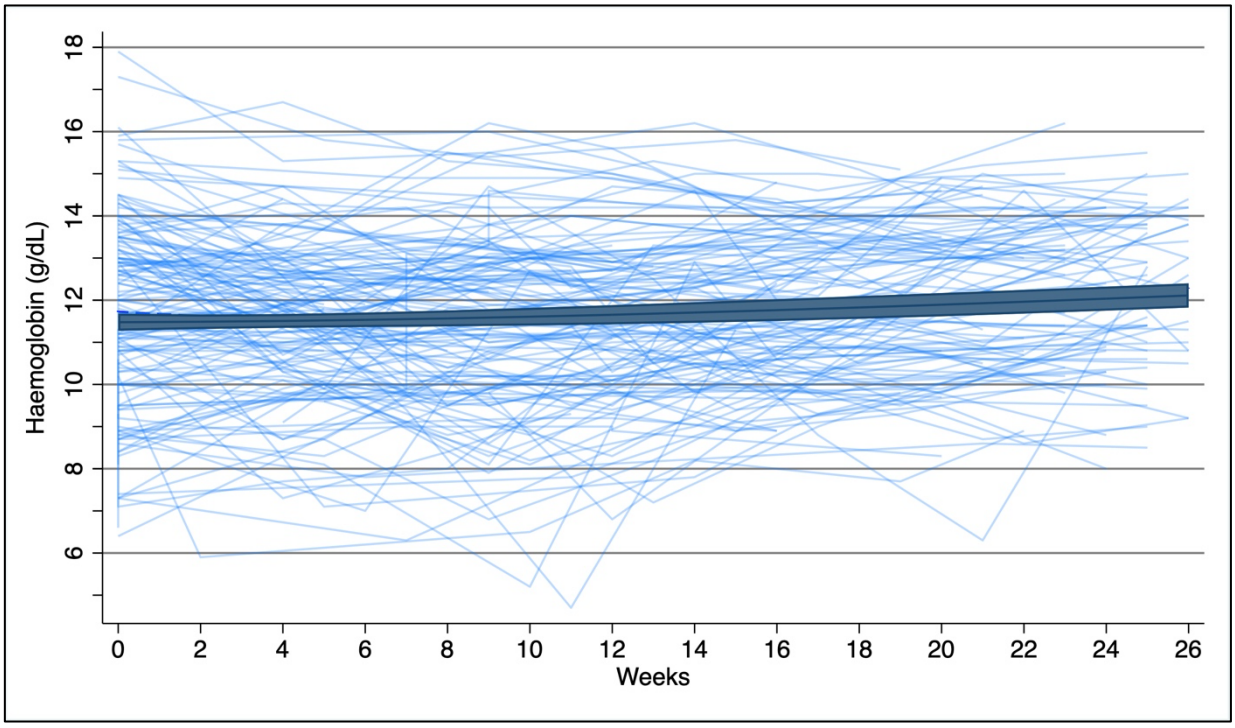


Figure 10 (A). Haemoglobin

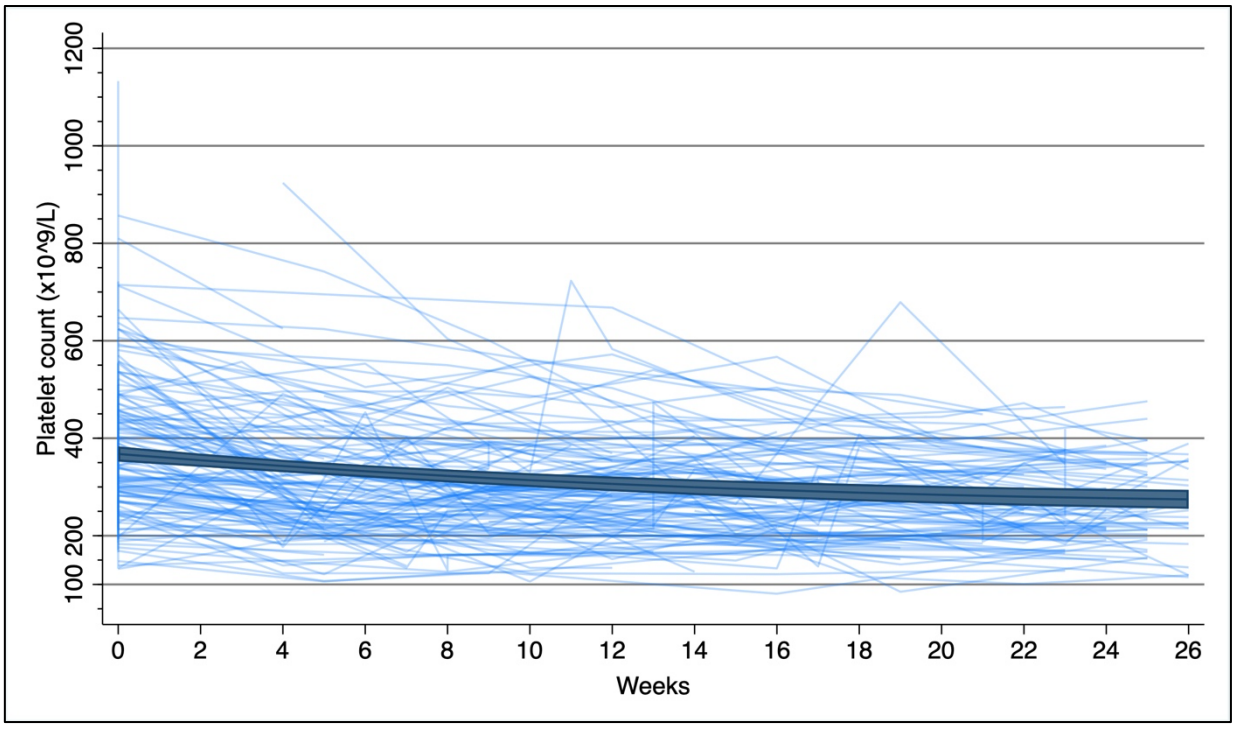


Figure 10 (B). Platelet count

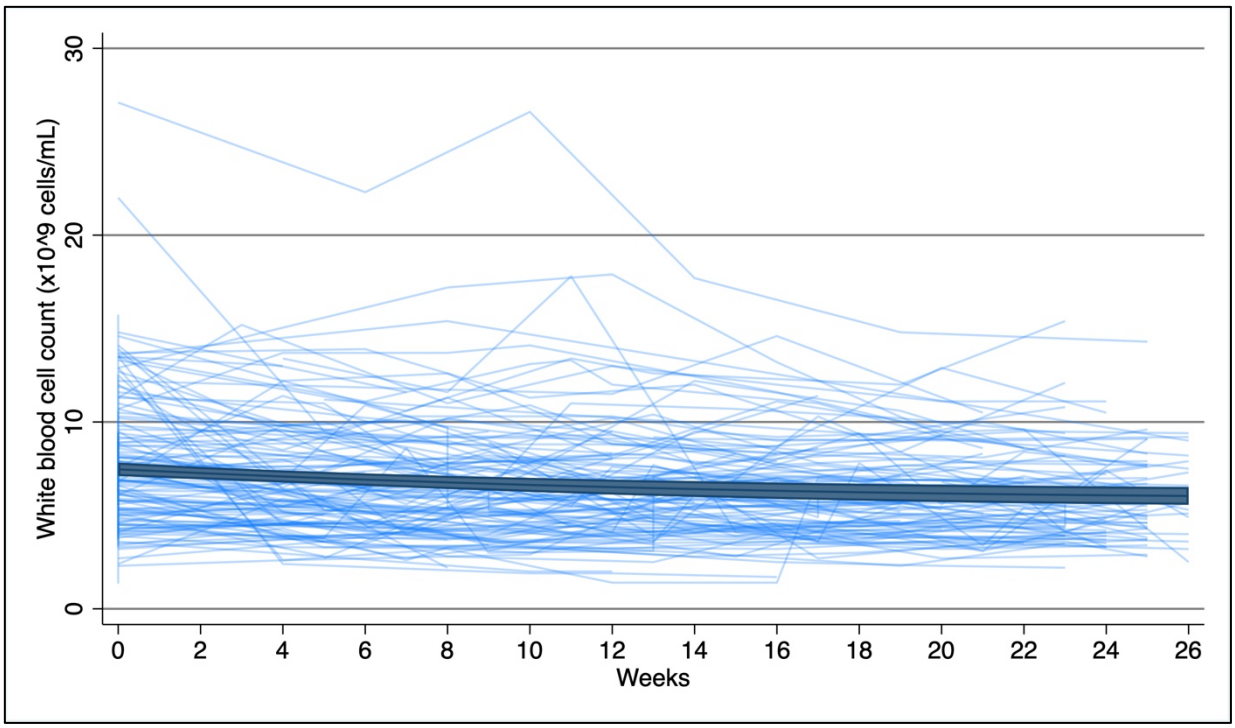


Figure 10 (C). White blood cell count

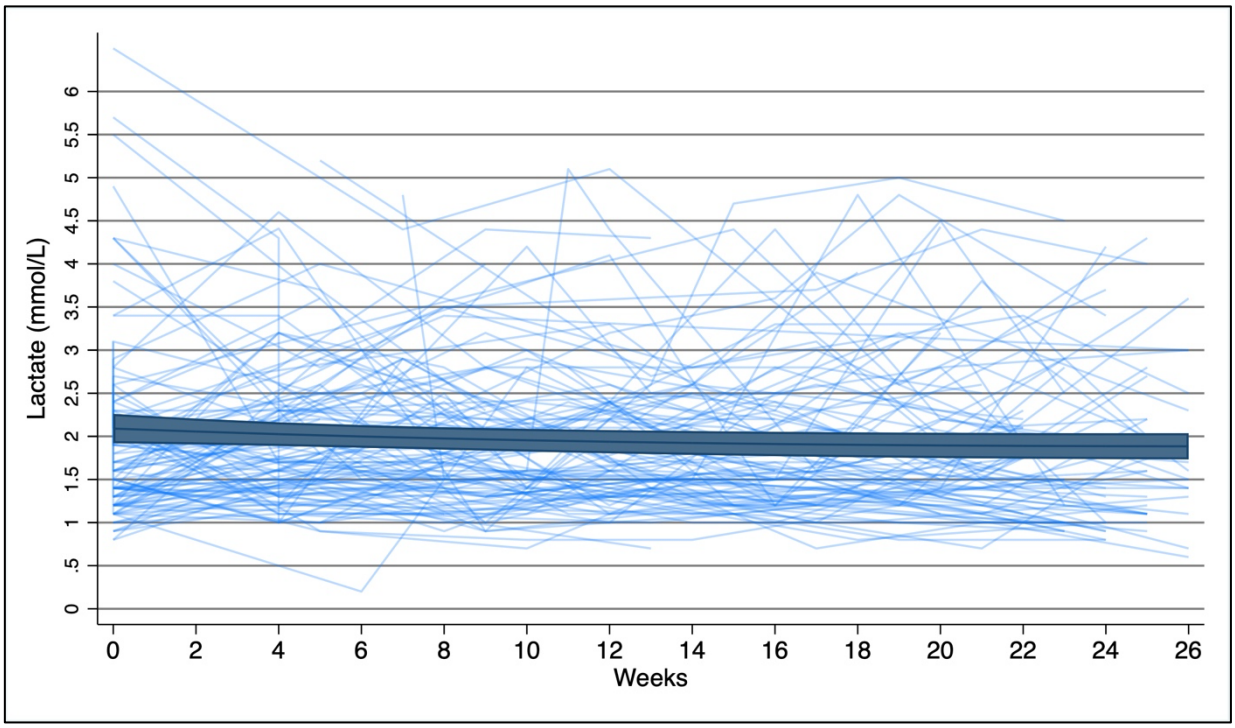


Figure 10 (D). Lactate

Figures 10 A - D. Teal lines indicate observed values, dark blue line indicates model-predicted trend (adjusted for covariates), blue shading indicates 95% confidence interval.

Factors independently associated with anaemia, defined as a reduction in haemoglobin > 2 g/dL, were linezolid trough concentration (aOR 1.4 per 1 mg/L increase, 95% CI 1.1

to 1.8), male sex (aOR 3.4, 95% CI 1.5 to 8.1) and age (aOR 1.7 per 10-year increase, 95% CI 1.2 to 2.3). HIV-positivity was not a significant predictor (aOR 1.2, 95% CI 0.5 to 2.9). There was large inter-individual variability (rho = 0.47). Marginal predictions for probability of anaemia are shown in Figure 5. A linezolid trough concentration ≥ 2.5 mg/L was associated with 2.9-fold increased odds (95% CI, 1.3 to 6.8) of anaemia in the adjusted model.

Figure 11. Predicted probability of anaemia by sex.

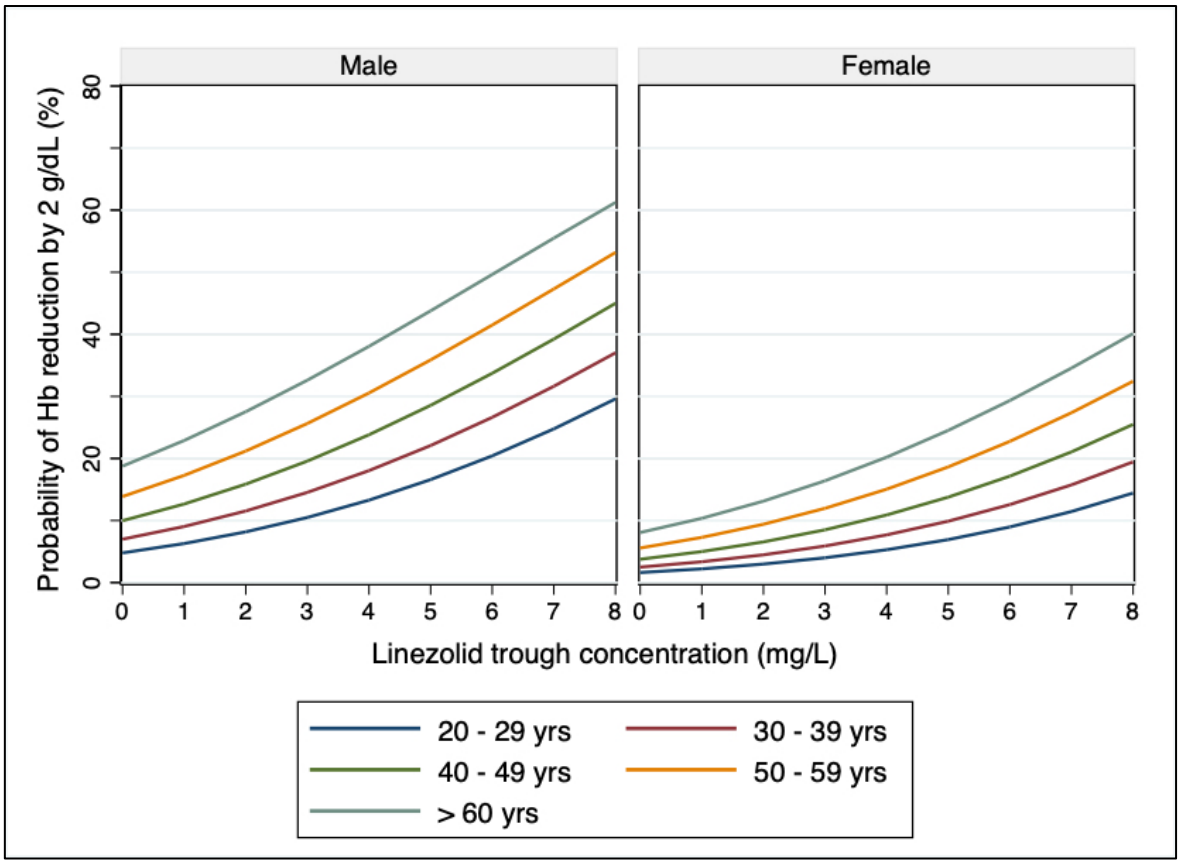


Figure 11. Marginal predictions from mixed-effects logistic regression model for probability of anaemia, defined as reduction in haemoglobin (Hb) ≥ 2 g/dL. Coloured lines indicate age ranges, defined in the legend.

There was also a significant association between linezolid trough concentration and thrombocytopenia and hyperlactatemia (Tables 6 and 7), but not with neuropathy (Table 8). There was no effect modification with inclusion of the mtDNA A2706G mutation in any model (data not shown). Model performance and parameter estimates were similar for all toxicity outcomes when AUC0-24 was tested instead of trough concentration (data not shown).

Table 6. Conditional logistic regression model outputs for thrombocytopenia, defined as reduction in platelet count by ≥ 250 x10^9/L.

Parameter	Adjusted odds ratio (95% confidence interval)	P-value
Linezolid trough concentration (per 1 mg/L increase)	1.4 (1.2 - 1.9)	0.004
Time on linezolid (per week)	1.0 (1.0 - 1.1)	< 0.001
Male sex	1.6 (0.3 - 8.1)	0.596
Age (per year increase)	1.0 (0.9 - 1.1)	0.926
HIV positive	0.5 (0.1 - 2.4)	0.397
Rho		0.755

Table 7. Conditional logistic regression model outputs for hyperlactatemia, defined as increase in lactate concentration by ≥ 1.5 mmol/L.

Parameter	Adjusted odds ratio (95% confidence interval)	P-value
Linezolid trough concentration (per 1 mg/L increase)	1.9 (1.3 - 2.6)	< 0.001
Time on linezolid (per week)	0.9 (0.9 - 1.0)	0.309
Male sex	0.5 (0.2 - 1.2)	0.121
Age (per year increase)	1.0 (0.9 - 1.0)	0.982
HIV positive	0.8 (0.3 - 1.7)	0.531
Rho		0.193

Table 8. Conditional logistic regression model outputs for peripheral neuropathy, defined as any new Grade increase on Brief Peripheral Neuropathy Score.

Parameter	Adjusted odds ratio (95% confidence interval)	P-value
Linezolid trough concentration (per 1 mg/L increase)	1.1 (0.9 - 1.3)	0.493
Time on linezolid (per week)	1.01 (1.00 - 1.03)	0.026
Male sex	2.1 (0.8 - 5.9)	0.145
Age (per year increase)	1.04 (1.0 - 1.1)	0.048
HIV positive	0.8 (0.3 - 2.2)	0.731
Rho		0.404

Discussion

In this cohort of South African RR-TB patients with an HIV prevalence of 63%, mild anaemia and peripheral neuropathy occurred frequently, and linezolid was prematurely discontinued in a fifth of patients. However, severe adverse events were infrequent, and on average, linezolid use in a multidrug regimen was associated with a positive treatment effect on haemoglobin over time. We identified a trough concentration threshold that predicted higher risk of anaemia, the most specific measure of linezolid toxicity, which, if validated, could be used for TDM.

Linezolid-associated haematological and neurological toxicity is a major concern for prescribers [80]. The most recent systematic review, published in 2015, summarized data from 14 retrospective studies and 1 randomized controlled trial; all but one study included fewer than 50 patients, and there was large heterogeneity in outcome definitions and treatment. The pooled proportion of adverse events leading to linezolid discontinuation was 29%, with anaemia and peripheral neuropathy reported in 31% and 27%, respectively [81]. Importantly, none of the included studies were conducted in Africa where high rates of HIV co-infection and limited monitoring capability may exacerbate the risk of linezolid toxicity [175]. A recent small prospective study (n = 63) among South African patients with RR-TB and a high HIV prevalence described similar proportions with anaemia and neuropathy at the 600 mg dose, with linezolid interruption or discontinuation in 35%, but severity was not reported, and it is unclear how outcomes were ascertained [245].

To obtain more reliable estimates of toxicity we defined haematological events using the established DAIDS grading system and used the validated BPNS scale to screen for peripheral neuropathy. We identified incident severe (Grade 3 or 4) adverse events in 15% of our participants. Anaemia followed by mild peripheral neuropathy were the most common adverse events, which is in line with other TB studies [41, 63]. Most adverse events occurred within the first 4 months of therapy, with similar timing of onset for anaemia and neuropathy at a median of around 10 weeks. Neuropathy has occurred relatively later than myelosuppression in some studies, leading to suggestions of a duration-dependent effect for neurotoxicity [63, 174, 192]. However, these studies were limited by small size and lack of consistent outcome definitions, and there is no clear biological explanation for this hypothesis. The onset of peripheral neuropathy in the Nix-

TB trial, which used a higher dose of linezolid, occurred mainly in the initial 3 months of treatment, consistent with our findings [41]. Cumulative incidence of reduced visual acuity was 12% at 24 months in our cohort, the earliest detected at 5 weeks after starting linezolid. This is within the range reported from other studies [81] but is likely an overestimate of true linezolid-induced optic neuropathy because visual acuity testing lacks specificity [259], and many participants were on concomitant ethambutol which can also cause ocular toxicity. In the Nix-TB trial optic nerve disorders were suspected in 11.9% by bedside testing but confirmed optic neuropathy only occurred in 2 (<2%) participants [41, 260].

There were no Grade 3 or 4 thrombocytopenia events in our cohort. Platelets are acute phase reactants, increasing in response to systemic inflammation, including from TB [261, 262], while haemoglobin changes in the opposite direction [263]. The negative correlation between platelet counts and haemoglobin over time in our data suggests that reductions in platelets represent reduction in systemic inflammation due to treatment rather than linezolid toxicity. Therefore, platelets are not a good pharmacodynamic marker for linezolid toxicity in tuberculosis.

We modelled change in haemoglobin as the primary toxicity outcome because of its relative specificity (a reduction is not expected with effective therapy) and large number of events. Random effects were included to account for latent (unmeasured) individual propensity to experience toxicity plus the interdependency among repeated observations within participants. Average haemoglobin increased over time after adjustment for other factors. HIV positivity was independently associated with reduced haemoglobin but not with anaemia (reductions > 2 g/dL). This effect was likely related to underlying HIV-related myelosuppression, as only 2 participants were on zidovudine. In the Nix-TB trial there was also no increase in linezolid-associated adverse events among HIV-positive participants [41]. Independent predictors of anaemia in our cohort were age, male sex, and linezolid trough concentrations, which have been associated with linezolid toxicity in patients with Gram-positive infections [109, 246]. In our cohort, the predicted probability of substantial haemoglobin reduction was ~10% for male participants at the median values of age and linezolid trough concentrations (Fig. 11), indicating relative safety of the 600 mg daily dose in our population. Despite this, linezolid was interrupted, dose reduced, or discontinued early in over half of

participants, suggesting either the presence of unmeasured adverse events or a low threshold by clinicians to alter or stop therapy due to concerns about toxicity potential [80].

Hyperlactatemia is a complication of linezolid therapy due to mitochondrial injury [184], and there have been case reports of lactic acidosis [189, 264]. Data is scarce on the incidence of hyperlactatemia in cohort studies. In the Nix-TB trial there were only 8 cases (3 had lactic acidosis), much lower than the 30% incidence in our study [41, 260]. Possible reasons for this discrepancy include technical issues relating to sample processing, a sicker population in our study, and different definitions of hyperlactatemia. Nonetheless, there were relatively few severe events, with only 12 Grade 2 episodes and 15% with increases > 1.5 mmol/L at 6 months; on average, lactate decreased over time on linezolid therapy.

The presence of SNPs at positions 2706 and 3010 in mitochondrial 16S rRNA have been reported in association with hyperlactatemia during linezolid therapy and are hypothesized to confer genetic susceptibility to linezolid toxicity through enhanced binding to mitochondrial structures [82]. The G3010A SNP was not detected in any of our participants and the presence of A2706G was not associated with any toxicity measure, corroborating findings from a trial among Korean DR-TB patients [83].

Linezolid is a good candidate for TDM in RR-TB because of its narrow therapeutic margin and large inter-individual variability [249, 251]. Linezolid trough concentrations are consistently associated with haematological toxicity measures [83, 100, 109, 247, 248], including in our cohort. A trough threshold of 2 mg/L has been suggested based on the high proportion of clinical events observed above that value among Korean XDR-TB patients in a small trial (n = 38) [83]. Although this target is now widely applied in PK/PD analyses, it has not been validated in other cohorts. Using a model-based approach we found that a range of trough concentrations around 2.5 mg/L described change in haemoglobin better than other tested values and had a large effect on risk of significant haemoglobin reduction after adjustment for other factors. Specificity of this value for predicting clinically significant anaemia was excellent (89%), but the sensitivity is poor. This suggests potential use for TDM to identify patients at risk of anaemia when trough concentrations exceed 2.5 mg/L, but not at lower values. Where TDM is

unavailable close clinical and haematologic (especially haemoglobin) monitoring could trigger linezolid dose reductions once toxicity develops [265].

There are limitations to consider when interpreting our findings. Other studies have linked linezolid interruption to adverse events, which may strengthen inferences about causality. We were unable to capture this relationship because of decentralisation of care in the TB programme. For the same reasons we did not measure resolution of peripheral neuropathy, but the low total number of severe events suggests progression was rare. There was no planned phlebotomy or neuropathy screening in the first month of our study, which may have contributed to the low event rate observed within the first few weeks of linezolid. Although we obtained all full blood count results from routine care, bedside haemoglobin testing was not captured, neither were blood transfusions, potentially masking more severe anaemia. However, a strength of our study is that it reflects real world practice and outcomes. The observational nature of the study resulted in unbalanced visits and missing observations, but random effects models are valid under flexible missing data assumptions, including missingness at random [266], supporting our conclusions. Linezolid concentrations were missing for a third of participants and individual drug exposures were predicted from a population PK model based on measured body weight and dose. The model was developed using rich data from a subgroup of participants our cohort and, on sensitivity analysis, inclusion of sparse concentrations did not alter model performance. A limitation of using trough values is that they are strongly influenced by other model parameters, plus uncertainty in dosing timing. We addressed this by including separate additive error and additive lag variability relative to reported time of the dose to account for uncertainty in unobserved dosing (affecting sparse samples) [251]. Additionally, there was no effect modification on parameter estimates when only values with observed concentrations were included in toxicity outcome models. Finally, our study was not formally powered, influencing the precision of our estimates and ability to detect relationships with smaller effects. There were relatively few observations above our identified toxicity concentration threshold of 2.5 mg/L, emphasizing the need to validate this finding. However, our sample size is larger than that used in previous studies which successfully identified PK/PD relationships for first-line TB treatment [267, 268] and for other studies evaluating linezolid toxicity [83, 245].

In summary, we characterized linezolid toxicity in a DR-TB treatment program among patients with high HIV prevalence. Severe events were uncommon at the standard dose of 600 mg daily in this setting and overall, linezolid use was associated with improvement haemoglobin and other toxicity measures. A trough concentration threshold of 2.5 mg/L should be further evaluated as a potential target for TDM of this important TB drug.

CHAPTER 5

Linezolid resistance in patients with drug-resistant tuberculosis and treatment failure in South Africa

ABSTRACT

Objectives: Limited data exist on clinical associations and genotypic correlates of linezolid resistance in *Mycobacterium tuberculosis*. We aimed to describe mutations and clinical factors associated with phenotypic linezolid resistance from patients with drug-resistant tuberculosis at two public sector facilities in South Africa.

Methods: Adults and adolescents with treatment failure (culture positivity ≥ 4 months) on a linezolid-containing regimen were retrospectively identified. Phenotypic resistance, as defined by a linezolid minimum inhibitory concentration (MIC) > 1 mg/L, was assessed on retrieved isolates using broth microdilution. Targeted sequencing of *rrl* and *rplC* was performed, irrespective of growth on subculture.

Results: Thirty-nine patients with linezolid-based treatment failure were identified, 13 (33%) of whom had phenotypic or genotypic linezolid resistance after a median duration of 22 months (range 7 - 32) linezolid therapy. Paired MIC testing and genotyping was performed on 55 unique isolates. All isolates with phenotypic resistance (n = 16) were associated with known resistance mutations, most frequently due to the T460C substitution in *rplC* (n = 10); *rrl* mutations included G2814T, G2270C/T, and A2810C. No mutations were detected in isolates with MICs at or below the critical concentration.

Conclusions: Linezolid resistance occurred in a third of patients with drug-resistant tuberculosis and treatment failure. Resistance occurred late and was predicted by a limited number of mutations in *rrl* and *rplC*. Screening for genotypic resistance should be considered for patients with a positive culture after 4 months of linezolid therapy in order to optimise treatment and avoid the toxicity of ineffective linezolid therapy.

Introduction

Drug-resistant tuberculosis has a major impact on health outcomes and costs in high-burden countries [25], and is expected to increase over the next two decades [14]. Linezolid, the prototype oxazolidinone, improved outcomes of drug-resistant tuberculosis in clinical trials [63, 174]. An individual patient data meta-analysis showed that linezolid use increased odds of treatment success 3-fold with a significantly lower mortality [38]. Based on these data, WHO recommended linezolid as a preferred agent for all patients with drug-resistant tuberculosis in 2018 [237]. Linezolid therefore has an important role as an antituberculosis agent and its introduction into national tuberculosis programs will be scaled up.

Linezolid resistance has been reported in clinical isolates from a limited number of patients with drug-resistant tuberculosis and treatment failure [63, 111]. Limited evidence suggests that population-level resistance to linezolid may be increasing in TB programs [202]. Linezolid shares key binding sites and displays cross-resistance with other oxazolidinones, including promising new agents in clinical development, such as sutezolid [113] and delpazolid [269]. Mutations in genes encoding the 23s rRNA (*rrl*) linezolid peptidyl transferase centre (PTC) binding site and the L3 protein (*rplC*), which extends into the binding site, have been identified as the dominant molecular mechanisms underlying linezolid resistance from *in vitro* and clinical studies [63, 113, 118, 121-124, 126, 269-272]. There are limited data on the association between genotypic and phenotypic linezolid resistance in clinical isolates. The few published studies describing linezolid resistance in treatment programs have not integrated MIC values with genotyping and have not explored important clinical parameters such as duration of linezolid exposure [272].

There are two potential risk factors for linezolid resistance. First, linezolid dosing is frequently reduced due to mitochondrial toxicity [238], which may lead to suboptimal exposures for efficacy and resistance suppression, driving the selection of resistant mutants [238]. Second, there are limited treatment options for drug-resistant tuberculosis and linezolid may be added to a failing or inadequate regimen, exacerbating the risk of acquired resistance.

A better understanding of the clinical predictors and genotypic correlates of linezolid resistance is critical to inform strategies to preserve this important antituberculosis agent. We conducted a retrospective cohort study of patients with drug-resistant tuberculosis and linezolid-based treatment failure at two tuberculosis referral hospitals in South Africa with the following objectives: (i) to determine the prevalence of linezolid resistance in this at-risk population, (ii) to identify the mutations associated with phenotypic linezolid resistance in clinical *Mycobacterium tuberculosis* isolates, and (iii) to describe clinical factors associated with linezolid resistance.

Patients and methods

Setting and study population

Adult and adolescent patients (≥ 13 years old) with treatment failure on a linezolid-containing regimen were retrospectively identified from two public sector tuberculosis facilities in South Africa: Jose Pearson Hospital in Port Elizabeth and Brooklyn Chest Hospital in Cape Town. These facilities manage both in- and outpatients with drug-resistant tuberculosis and use linezolid routinely in their treatment regimens for pre-XDR (defined as resistance to rifampicin and isoniazid, plus fluoroquinolones or second line injectables) and XDR-TB (as for pre-XDR but with resistance to both fluoroquinolones and second line injectables). Treatment failure, and eligibility for inclusion in the analysis, was defined as a persistently positive sputum culture for *M. tuberculosis* or culture reversion after a negative culture in a patient who had received at least 4 months of linezolid-based therapy for tuberculosis.

Clinical cases

Registers of patients with possible linezolid-based treatment failure are maintained by facility staff members; these medical records were screened by a study investigator. Clinical data were extracted and captured directly onto electronic case report forms in REDCap [273]. The index tuberculosis episode was defined as receipt of continuous treatment (with < 3 months' interruption) for rifampicin-resistant tuberculosis. We quantified the number of likely effective agents in the regimen by applying a scoring system according to resistance profile, prior exposure, and known clinical effectiveness (Table S1) [274].

Table S1. Rules for rating likely effectiveness of drugs in background regimen.

Drug	Context/rationale	Score
Pyrazinamide	Baseline resistance in MDR/XDR TB is ~60% [275]	0.5
Fluoroquinolones	XDR or pre-XDR with FQ resistance All other circumstances	0 1
Injectable agents	XDR or pre-XDR with injectable resistance All other circumstances	0 1
Ethambutol	Baseline resistance in MDR/XDR TB is ~45% [275]	0.5
Isoniazid	inhA mutation Dual inhA/katG mutations or katG mutation alone	0.5 0
Ethionamide	Dual inhA/katG mutations or inhA mutation alone All other circumstances	0 1
Clofazimine	If previous XDR or pre-XDR-TB treatment (likely exposed) No previous XDR or pre-XDR-TB treatment (no exposure)	0.5 1
PAS	If previous XDR or pre-XDR-TB treatment (likely exposed) No previous XDR or pre-XDR-TB treatment (no exposure)	0.5 1
Terizidone	If previous MDR-TB treatment (likely exposed) No previous MDR-TB treatment (no exposure)	0.5 1
Macrolides	No clinical efficacy	0
Amoxicillin-clavulanate	No clinical efficacy	0
Bedaquiline	No previous exposure	1
Rifabutin	No previous exposure, and susceptible by genotyping	1
Delamanid	No previous exposure	1

Table S1. Molecular and/or phenotypic drug susceptibility testing results from routine testing were available for rifampicin, isoniazid (including the presence of *inhA* and *katG* mutations), fluoroquinolones, and second line injectables for at least one isolate from each patient.

Microbiological data

Sputum culture results from routine testing performed at the study sites are linked to the National Health Laboratory Services (NHLS) database, which was used to identify *M. tuberculosis* isolates from identified cases. All previous isolates were requested from both local NHLS laboratories and from the National Institute of Communicable Diseases (NICD), which on clinician request performs extended drug susceptibility testing (DST). Available isolates were shipped in original liquid culture bottles to the BSL3 laboratory at the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town for linezolid resistance testing.

Isolate selection and culture conditions

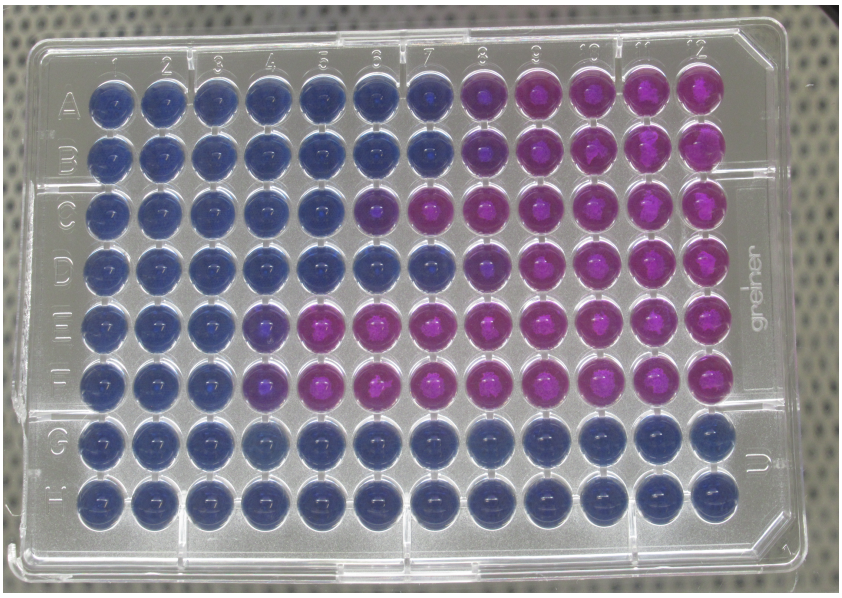
Subculture was done for all samples from the first batch of retrieved isolates (n = 57); in subsequent batches, only paired isolates (the earliest and most recent) from each patient with linezolid-based treatment failure underwent subculture (n = 46). *M. tuberculosis* isolates were initially cultured in the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system (Becton, Dickinson and Company, Sparks, MD, USA) according to the manufacturer's instructions. Subsequently, 100 μL of each MGIT culture was inoculated onto Lowenstein-Jensen (LJ) medium slants (Becton, Dickinson and Company, Sparks, MD. USA) and incubated at 37°C for 4-6 weeks with continuous aeration. Colonies were scraped from LJ slants with visible bacterial growth and 10% glycerol stocks were made. Subcultures were initiated by inoculating 100 μL of the 10% glycerol stock in 10 mL Middlebrook 7H9 broth (Sigma-Aldrich) supplemented with 0.2% (v/v) glycerol, 0.1% Tween 80 and 10% (v/v) OADC and were incubated at 37°C until an optical density at 600 nm (OD_{600}) value of 1 was reached.

Determination of linezolid minimum inhibitory concentration (MIC)

Phenotypic linezolid resistance was determined by MIC assessment using a resazurin microtitre assay [131]. Two-fold serial dilutions of linezolid (range 64 mg/L to 0.0625 mg/L) were made in 7H9 medium supplemented with 0.1% casitone, 10% OADC and 0.5% glycerol in 96-well U-bottomed plates. The enriched 7H9 broth containing retrieved *M. tuberculosis* isolates was diluted 1:1000 and inoculated into the linezolid-containing plates. Plates were incubated at 37°C for 14 days before adding 20 μL of 0.025% (w/v) resazurin (Sigma-Aldrich), followed by incubation for an additional 24 to 48 hrs. The MIC value was defined as the lowest linezolid concentration that inhibited

growth, indicated by a colour change from blue to pink (Figure 1). Positive (*M. tuberculosis* isolate only) and negative (7H9 medium only) controls were included for each assay. Phenotypic resistance was defined by an MIC > 1 mg/L, the recognized critical concentration for linezolid [240].

Figure 1. Microtitre assay for linezolid MIC determination.



DNA extraction, PCR amplification and sequencing

DNA was extracted from all MGIT cultures, including those without growth on LJ slopes, using the Chelex method [276]. Primers were designed to amplify coding and flanking regions for *rrl* as well as an 814 bp product covering *rplC* (Table S2). These targets were selected because they encode regions in or near the 23s rRNA binding site [197, 277], and have been associated with linezolid resistance in clinical and laboratory-generated *M. tuberculosis* isolates [278]. We also planned to sequence *rplD* (which encodes a putative resistance target in the L4 protein) [269] in isolates with MIC > 1 mg/L and no detectable mutations in *rrl* and *rplC*, but this was not required. Primer design was based on the genome sequence of the *M. tuberculosis* H37Rv reference strain (http://genolist.pasteur.fr/TubercuList) and performed using Primer 3 software version 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/).

Table S2: Primer sets used for the amplification and sequencing of *rplC, rrl* and *rplD.*

Primer	Sequence 5' to 3'	Fragment length (bp)

rplC F	CACAAGCGGTTGATCGACAT	814
rplC R	GCGTCTTGACGTCGATTTTG	
rrl (1) F	AGTTGGCCACCAACACACTG	944
rrl (1) R	TGGGTCGCCCTATTCAGACT	
rrl (2) F	GGTTAACCCGTGTGGGGTAG	1101
rrl (2) R	TTCTTGGCAGCAGAGGATCA	
rrl (3) F	CGATGGACAACGGGTTGATA	1016
rrl (3) R	GGCGCCTCCGTTACATTTTA	
rrl (4) F	CGAAATTCCTTGTCGGGTAAG	1017
rrl (4) R	ACGGATGTGGTTGCGAGTTT	
rplD F	TTGGTGCATAAGGTCGATGC	941
rplD R	TGACGGCAAAAATCTTCTCG	

PCR reactions were performed under the following thermocycling conditions: 15 minutes (min) denaturation at 95^{o}C followed by 35 amplification cycles (each cycle: 94^{o}C for 1 min, 62^{o}C for 1 min, 1 min extension at 72^{o}C) and the final elongation step of 10 min at 72^{o}C. Successful PCR amplification was confirmed by gel electrophoresis. PCR products underwent Sanger sequencing at Central Analytical Facilities, Stellenbosch University, South Africa. Mutations were detected using CLC Main Workbench, Version 7.7.3 (Qiagen, CA, USA) by aligning the reference H37Rv strain (ATCC 27294) sequence to the sequence from the clinical isolates. Genotypic resistance was defined as the presence of single nucleotide substitutions in *rrl* or *rplC* previously identified to be associated with linezolid resistance,[278] as well as newly-identified polymorphisms in close proximity to the linezolid binding pocket and associated with elevated MICs.

Analysis

MIC distributions of isolates that underwent phenotypic DST were plotted. We used bivariate analysis to compare demographic profile, treatment history, and linezolid exposures between patients who developed linezolid resistance (phenotypic or genotypic) with those who did not. Wilcoxon rank-sum testing was performed for comparisons of continuous variables and χ^2 tests for categorial variables. A Kaplan-Meier survival plot was constructed for time to the detection of linezolid resistance, censored for death, loss to follow-up, and at 36 months post-linezolid initiation.

Ethics

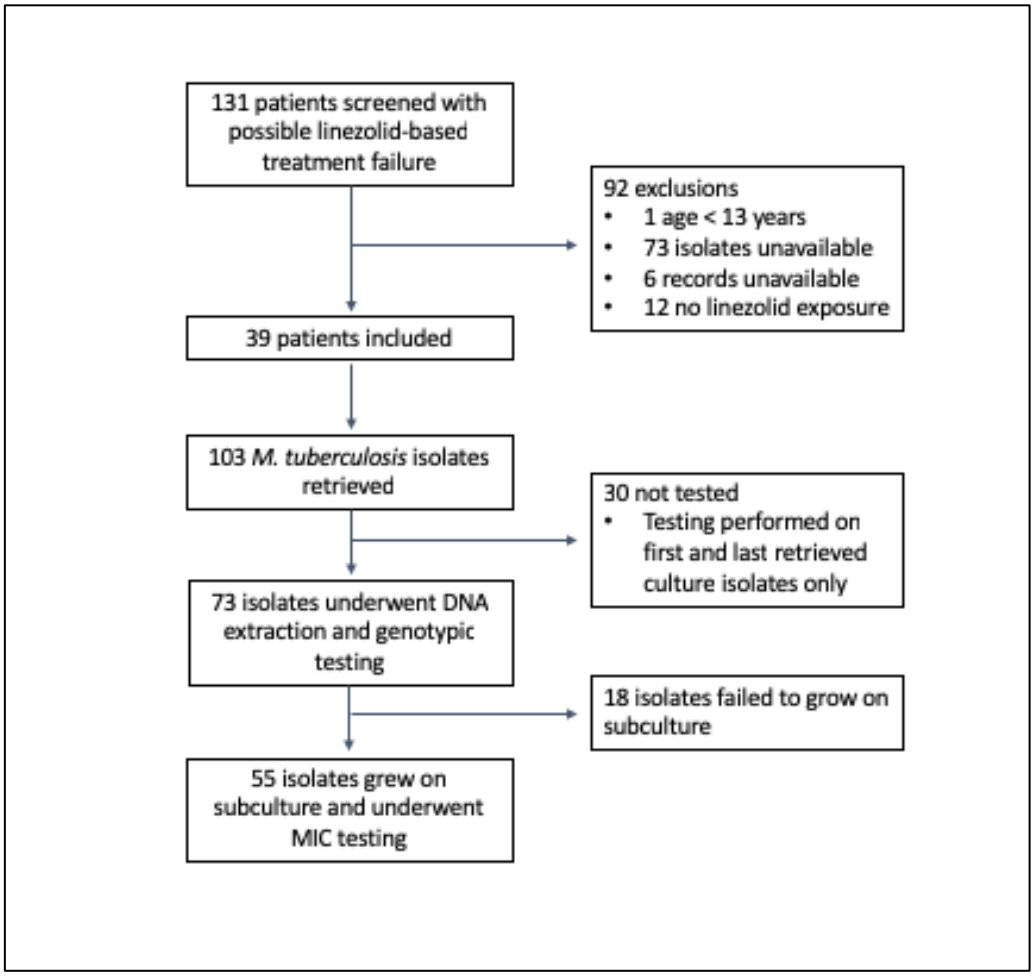
This study was approved by the University of Cape Town Human Research Ethics Committee (reference 805/2016); the requirement for informed consent was waived for collection and analysis of stored *M. tuberculosis* isolates and retrospective clinical data.

Results

Linezolid MIC distribution and associated resistance mutations

We screened 131 patients with drug-resistant tuberculosis and suspected linezolid-based treatment failure (Figure 2); 103 *M. tuberculosis* culture isolates were available from 39 eligible patients (34 in Port Elizabeth and 5 in Cape Town) collected between May 2010 and September 2017.

Figure 2. Flow diagram showing numbers of patients and isolates included.



Paired MIC testing and genotyping was performed on 55 unique isolates that grew on subculture, demonstrating a clear bimodal distribution around the critical concentration of 1 mg/L (Figure 3). All isolates with MIC > 1 mg/L (phenotypic resistance, n = 16) were associated with known resistance mutations in either *rrl* or *rplC*; conversely, no resistance-conferring mutations were detected in isolates with MICs at or below the critical concentration (Table 1).

Figure 3. Distribution of *M. tuberculosis* linezolid MIC values for 55 clinical isolates with paired MIC and sequencing results.

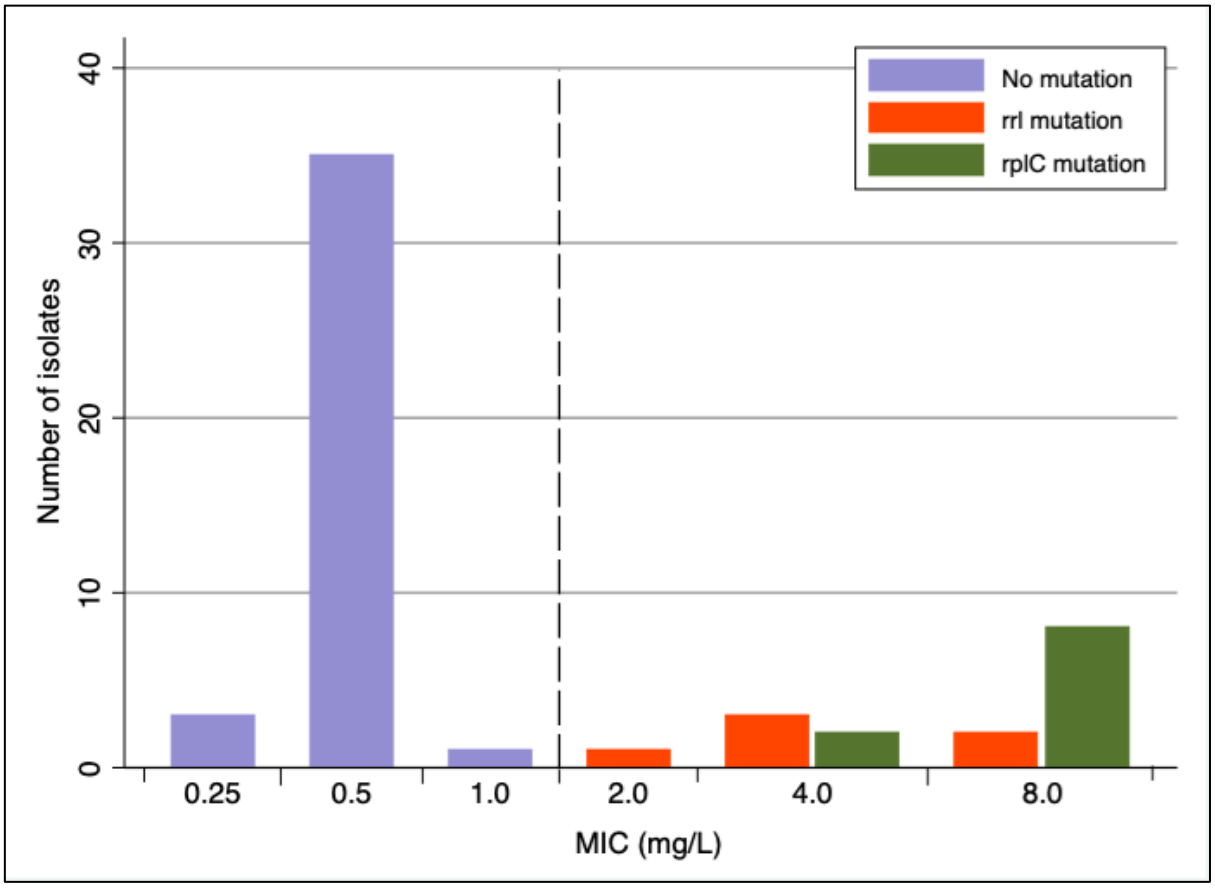


Figure 2. The vertical dashed line represents the critical concentration value for linezolid (1 mg/L). None of the isolates had dual mutations.

Table 1. Mutations in *rrl* and *rplC* with corresponding MIC values detected from all retrieved *M. tuberculosis* isolates that underwent phenotypic and/or genotypic resistance testing (n = 73)

Participant ID	Isolate number	Duration on linezolid, months*	Linezolid MIC (mg/L)	*rrl* mutation	*rplC* mutation
2007	XD00813360	10	8	**G2814T**	WT

				[2576]	
1007	UH00774544	18	4	**(G2270T) [2032]**	WT
1008	UH00806598	25	8	WT	**T460C**
1010	YA00027930	12	2	**(G2270C) [2032]**	WT
1011	UH00751414	23	4	**G2814T [2576]**	WT
	UH00768146	24	4	**G2814T [2576]**	WT
1013	UH00760075	13	8	WT	**T460C**
	UH00830976	18	4	WT	**T460C**
1014	UH00812719	22	8	**G2814T [2576]**	WT
1015	UJ00479546	8	8	WT	**T460C**
	UJ00506756	10	8	WT	**T460C**
	UJ00519199	11	8	WT	**T460C**
1023	UH00754483	13	No growth[#]	A2384C [2146]	**T460C**
	TRL0118476	5	8	WT	**T460C**
1032	UH00873025	26	4	WT	**T460C**; G546A
1043	TRL0118350	10	8	WT	**T460C**
1050	UH00962529	23	8	WT	**T460C**
1057	UH00820877	25	No growth[#]	**A2810C [1942]**	WT

Table 1. Nucleotide positions are given according to the sequence of *M. tuberculosis* strain H37Rv (GenBank accession No. NC_000962.3) with corresponding *E. coli* positions reported in [square] brackets below. Mutations shown in parentheses were identified in the heteroresistant state. WT, wild type. *Linezolid exposure from time of initiation to collection of the isolate. [#]No growth in LJ culture

Single nucleotide polymorphisms associated with phenotypic resistance to linezolid

Sequencing of both *rplC* and *rrl* was done on 73 unique isolates (including isolates that failed to grow on subculture) from the 39 clinical cases with linezolid-based treatment failure. Mutations and corresponding MIC values from 13 patients (18 isolates) with phenotypic and/or genotypic resistance are listed in Table 1. Resistance mutations in

rplC (n = 11) were detected more frequently than in *rrl* (n = 7); none of the isolates harboured dual resistance-conferring mutations. The 2814G→T substitution was the most frequently detected mutation in *rrl* (present in 4 out of 7 isolates), followed by point mutations in two isolates at position 2270 (G → C/T) and one isolate with a A2810C mutation. The G2270C/T alleles were present with wild-type alleles as mixed populations from two unique patients, and were not detected in strains recovered 1 and 6 months earlier, respectively (Figure 4). We detected the following additional polymorphisms in *rrl*, which were not considered to represent resistance mutations due to distance from the PTC and because they were not associated with elevated MICs (MIC 0.25 mg/L for all): A2384G (n = 3), A2384C (n = 5), G2399A (n = 1), and mixed G2399A/A2384C (n = 3).

Figure 4. Timing of linezolid initiation and results of phenotypic and genotypic testing in relation to start of antituberculosis therapy for three patients with sequential isolates demonstrating evolution of linezolid resistance.

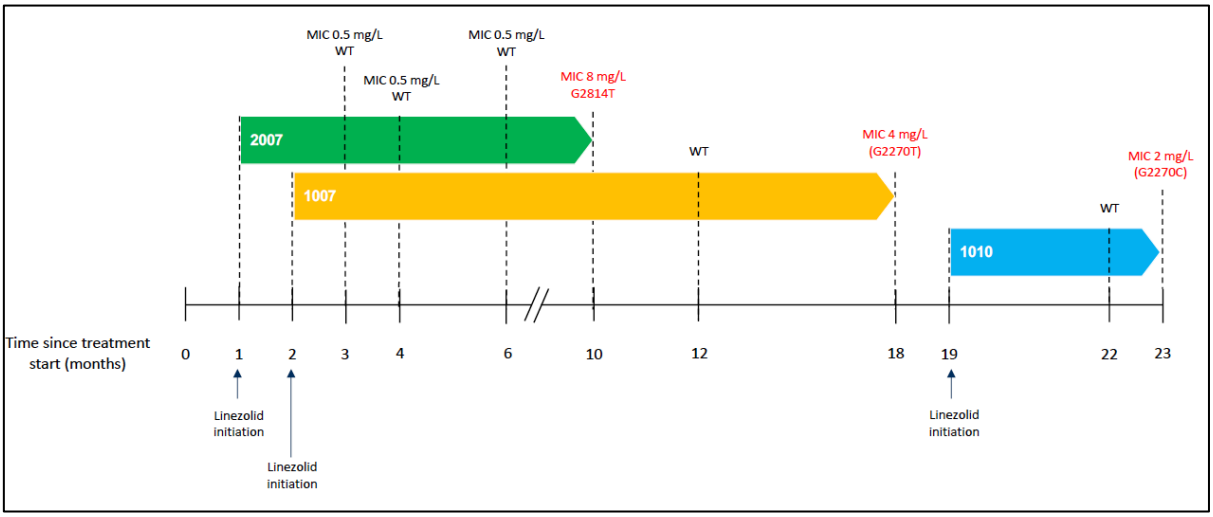


Figure 4. WT, wild type; MIC, minimum inhibitory concentration. Inset numbers are study patient identifiers. Mutations shown in parentheses were identified in the heteroresistant state.

rplC resistance mutations (n = 11) were exclusively due to T460C. We found a non-resistance conferring polymorphism G546A (MIC 0.5 mg/L) in two isolates: as a single mutation in one isolate and mixed with T460C in another isolate with an elevated MIC. A GCC insertion at position 466 was identified in one isolate which failed to grow on subculture and consequently no MIC result was available; however, this insertion was not detected in a sequential isolate collected 6 months later.

Clinical characteristics of the study population

Demographic and clinical characteristics of all included cases with linezolid-based treatment failure are shown in Table 2, disaggregated by the presence of linezolid resistance. Overall, twenty-three (61%) patients were HIV-positive, and the majority (n = 28, 72%) had XDR-TB. There were a median of 4 (3.5 – 5) likely effective agents in addition to linezolid at the time of treatment failure; only 8 (21%) patients had isolates that were fully susceptible to fluoroquinolones, and bedaquiline and/or delamanid were included in the regimen for only 9 (23%) patients. Linezolid initiation was delayed for a median of 7 months (IQR 2 – 17, range 1 – 30) after the start of therapy for the index tuberculosis episode and was administered for a median of 16 months (IQR 12 – 23, range 5 – 44) until the last obtained culture result. The standard dose for linezolid was 600 mg daily, reduced to 300 mg daily in 20 (51%) patients.

Table 2. Clinical characteristics of patients with linezolid-based treatment failure.

	Resistant n = 13	**Susceptible n = 26**	**P-value**
Age, years	35 (30 – 45)	36 (28 – 42)	0.83
Male sex	6 (46)	13 (50)	0.82
Weight at treatment initiation, kg	48 (39 – 62)[a]	45 (35 – 54)[b]	0.32
HIV positive	7 (54)	16 (64)	0.54
Number of previous TB episodes	1 (1 – 2)	1 (1 – 2)	0.41
Baseline resistance pattern – MDR – Pre-XDR (Inj) – Pre-XDR (FQ) – XDR	 1 (8) 1 (8) 0 (0) 11 (85)	 1 (4) 5 (19) 3 (12) 17 (65)	 0.40
Delay in linezolid start after initiation of therapy, months	8 (2 – 13)	3 (0 – 9)	0.24
Record of poor adherence	6 (67)	12 (67)	1.0

Linezolid dose reduction	6 (46)	14 (61)	0.39
Duration on linezolid, months[c]	18 (10 – 23)	16 (12 – 21)	0.89
Number of other drugs	10 (9 – 11)	8 (7 – 10)	0.04
Number of likely effective drugs at time of treatment failure	4.0 (4.0 – 4.5)	4.0 (3.5 – 5.0)	0.95
Bedaquiline exposure[d]	3 (23)	9 (35)	0.46
Duration of bedaquiline exposure, months[e]	5 (1 – 10)	10 (6 – 12)	0.19
Outcome within 48 months of study			
– In care	6 (46)	8 (31)	0.55
– Died	6 (46)	9 (35)	
– LTFU	0 (0)	2 (8)	
– Palliation	0 (0)	3 (12)	
– Unknown	1 (8)	3 (12)	

Table 2. Data are n (%) or median (IQR). Resistant is defined as MIC > 1 mg/L and/or presence of previously published resistance-conferring mutation.

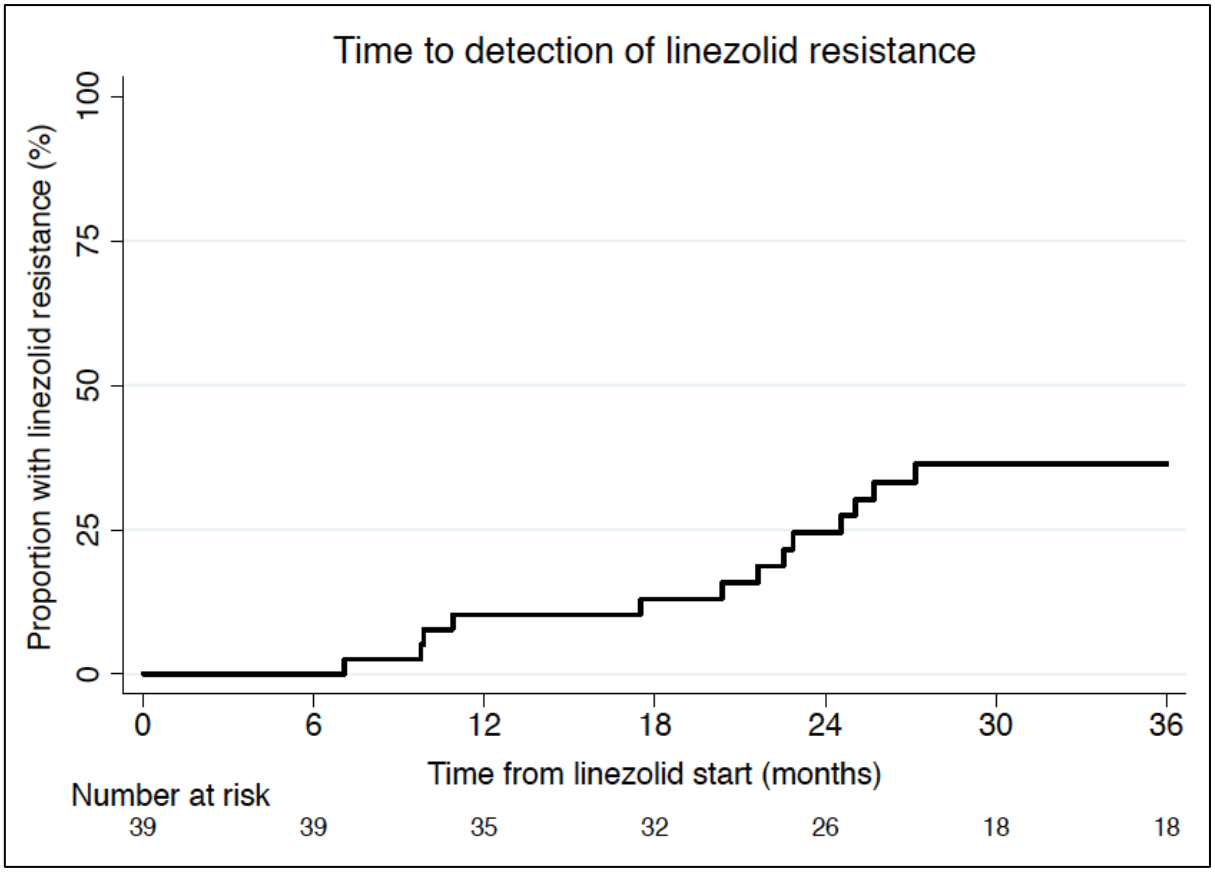
a. n = 12
b. n = 23
c. Defined as the time from linezolid initiation until the first culture showing linezolid resistance or the last culture obtained in those without linezolid resistance (n = 39).
d. The number of patients with bedaquiline exposure before the first culture showing linezolid resistance or the last culture obtained in those without linezolid resistance.
e. Defined as the time from bedaquiline initiation until the first culture showing linezolid resistance or the last culture obtained in those without linezolid resistance (n = 12).

Clinical associations with linezolid treatment failure and resistance

Linezolid resistance was detected by either phenotypic or genotypic methods in 13 (33%, n = 39) patients with linezolid-based treatment failure. The earliest detected occurrence of resistance was 7 months after initiating linezolid, with the latest at 27 months (Figure 5). Neither linezolid dose reduction (P = 0.39) nor overall duration (18 months for those with resistance versus 16 months without; P = 0.89) were associated with linezolid resistance (Table 2). Bedaquiline exposure prior to treatment failure did

not appear to be protective for linezolid resistance in this cohort; 3 (23%) with resistance versus 9 (35%) without resistance received bedaquiline; P = 0.46. The only significant difference between those with and without resistance on bivariate analysis was the total number of antituberculosis drugs received, which was higher in the group with linezolid resistance (median 10 versus 8 drugs, P = 0.04). There was trend towards a longer delay in linezolid initiation in the group with resistance (8 months versus 3 months), but this was not significant (P = 0.24). Overall mortality was 38% (n = 15) during the 42-month observation time.

Figure 5. Kaplan-Meier plot showing time to detection of linezolid resistance, censored for death and loss to follow up (n =39).



Discussion

We retrospectively identified 39 patients with drug-resistant TB and linezolid-based treatment failure from two geographically distant treatment facilities in South Africa. Most patients had XDR-TB and were unable to obtain early access to new antituberculosis agents. Consequently, background drug regimens at the time of linezolid introduction were likely suboptimal resulting in effective linezolid monotherapy

for prolonged periods of time. Linezolid resistance was detected in over 30% of individuals and only after a median of 18 months of exposure, which is somewhat surprising given the highly conducive conditions for resistance selection. In one respect this observation is reassuring, because it confirms linezolid's high barrier to resistance seen under similar conditions in a clinical trial [63] as well as *in vitro* where very low mutation frequencies are achieved relative to other antituberculosis drugs [113, 118]. However, our finding also illustrates a fundamental reality underlying the large-scale expansion of linezolid for tuberculosis treatment: despite its high barrier to resistance, with sufficient selection pressure the emergence of linezolid resistance in tuberculosis treatment programs is inevitable. Of concern, there have been suggestions of a trend towards increasing population-level resistance in countries with a long history of linezolid use [202]; and linezolid resistance has been associated with the Beijing genotype [121], the dominant circulating *M. tuberculosis* strain in the Eastern Cape Province [32] where most of our cases were identified. Increased vigilance and active surveillance are clearly needed as linezolid is introduced into national tuberculosis treatment programmes.

Published data on linezolid resistance from patients with tuberculosis are scarce; in a literature review we identified nine studies that investigated linezolid resistance in clinical isolates, reporting on a total of 24 unique patients. Our study, involving 39 patients with linezolid-based treatment failure, likely provides the largest and most detailed series linking MIC values with molecular testing and clinical and treatment data. There are several notable findings which build on existing knowledge in this area.

Our strategy to perform targeted sequencing of *rrl* and *rplC* was based on the mechanism of linezolid action and observations from clinical and *in vitro* reports. It is unsurprising that mutations in 23s rRNA, particularly in proximity to the PTC binding site, predictably lead to MIC elevations and clinical resistance. The most frequently reported mutation in *rrl*, the 2814G $\rightarrow$T nucleotide substitution [63, 113, 115-117, 272], was detected in isolates from more than half of patients with linezolid-resistance and *rrl* mutations, and associated with MIC values of up to 8 mg/L. We also detected G2270C/T mutations in isolates from two unique patients which, to our knowledge, is the first report in clinical strains. These mutations were associated with lower MICs (2 - 4 mg/L) in our cohort (as well as in previous *in vitro* studies [118, 271]) which may be related to the

position outside the PTC and the fact that in both cases the mutations were identified in the presence of the susceptible allele (heteroresistant state). We did not detect the resistance allele in prior isolates from either of these patients (collected 1 month and 6 months earlier, respectively), suggesting the evolution of linezolid resistance with ongoing selection pressure.

The third *rrl* mutation we identified, A2810C, has been previously described in isolates from patients with treatment failure [115, 116]. Although we were unable to determine the MIC associated with this mutation, this nucleotide substitution is in relative proximity to the PTC and its previous detection in isolates with phenotypic resistance suggest that it could confer a linezolid resistance phenotype.

Overall, the T460C mutation in *rplC* was the underlying cause for linezolid resistance in the majority (7/13, 54%) of our cases; its dominance has also been noted in other settings [113, 118, 123, 270-272]. This mutation results in an amino acid exchange from cysteine to arginine at position 154 in the L3 protein that extends into the linezolid binding site [277], resulting in MIC ranges of 2 – 32 mg/L [63, 113, 118, 121-124, 126, 269-272]. The *rplC* T460C mutation has been associated with lower MICs and a lower fitness cost [113], than mutations affecting 23s rRNA. Interestingly, in our cohort, the converse was found, with higher MICs linked to *rplC* mutations; this has also been described for *in vitro* mutants [118, 271], reinforcing the importance of this key mechanism for linezolid resistance. It is difficult to interpret the significance of the GCC insertion at position 466 found in one isolate because we were unable to determine the MIC; this has not been previously associated with linezolid resistance, including in bacteria other than *M. tuberculosis*, and is likely not to be resistance-conferring.

Genotyping had excellent accuracy and discriminative value for predicting phenotypic resistance (MIC > 1 mg/L) in the 55 isolates with both sequencing and MIC results. It appears that sequence mixes in *rrl* and *rplC* have low diversity and there are a limited number of mutations that underlie linezolid resistance. This raises the possibility of translation into rapid molecular diagnostics, which are needed to support linezolid rollout into national tuberculosis programs where phenotypic testing is not widely available. Furthermore, molecular testing could be class-based because of cross resistance with

other oxazolidinones [113, 123, 269], and has the advantage of detecting low-frequency mutations supporting early identification of resistance.

We found that the number of background agents to which patients were exposed during the index tuberculosis episode was significantly higher amongst those with linezolid resistance. This could reflect a tendency of clinicians to add more drugs to failing regimens, leading to a paradoxically higher risk of linezolid monotherapy with more background drugs. The observed trend in longer delays to linezolid initiation in patients with linezolid resistance supports this. Although there is no direct cross-resistance between linezolid and other antituberculosis drug classes, one study has demonstrated an association between linezolid MIC elevations and the use of other second-line antituberculosis drugs, specifically fluoroquinolones and kanamycin.[202] This may be due to induction of efflux pump expression from antimycobacterial drug exposure which initiates a pathway leading to subsequent high-level mutation-related resistance [279]. Regardless of the presence of linezolid resistance there was an extremely high mortality amongst this cohort of patients with linezolid-based treatment failure. This emphasizes the need for early inclusion of new drugs such as bedaquiline to strengthen treatment regimens and reduce the risk of treatment failure [280] and mortality [60].

Our retrospective study had a number of important limitations. By definition, we had to rely on data that were collected in the clinical service and not originally intended to address the aims of this study. We therefore had to accept risks of major biases when describing and comparing patients with linezolid-based treatment failure. We are confident, however, that our strategy to screen and identify cases from hospital registers was sufficiently rigorous to avoid excluding important outliers. The accuracy of clinical data extracted from medical records was imperfect and also may have influenced the robustness of our findings, particularly in relation to antituberculosis drug exposures, treatment adherence, and assessment of regimen effectiveness. However, the key parameters of linezolid duration and bedaquiline use were well documented. A substantial proportion of isolates were either not available from local laboratories (due to being lost or discarded; 73/131 screened patients, 56%) or were not viable on subculture (18/73 retrieved isolates, 25%). This has two potential consequences. First, sequential isolates demonstrating the transition to linezolid resistance were not available for the majority of included patients. The time-to event analysis could therefore

have overestimated the delay in the development of linezolid resistance. Second, isolates with linezolid resistance may be associated with fitness cost [113] and failure to grow, biasing our results. To address this, we sequenced all isolates, regardless of culture viability, and found a resistance mutation in only two isolates without MIC data. Sanger sequencing itself has imperfect sensitivity, particularly for the detection of mixed strain genotypes [281]; it is possible that we may have identified additional or novel mutations using next generation sequencing [282], an approach that should be considered in future studies.

In conclusion, we have shown that linezolid resistance occurred in a third of patients from this high-risk cohort in South Africa. Phenotypic resistance was detected late and was predicted by a limited number of mutations in *rrl* and *rplC*. Screening for genotypic resistance should be considered for patients with a positive culture after 4 months of linezolid therapy in order to optimise treatment and avoid the toxicity of ineffective linezolid therapy.

CHAPTER 6

Conclusions

Until recently, treatment for RR-TB required a reversion to pre-rifampicin regimens with toxic and poorly effective drugs administered over at least 18 months of therapy. Consequently, treatment outcomes have been dismal, with devastating impacts on affected communities and driving ongoing transmission. Introduction of new and repurposed drugs has been transformative, enabling shorter all-oral regimens and treatment outcomes that compare favourably to drug-susceptible TB. Linezolid has been a cornerstone of this treatment revolution and is likely to remain a key component of future RR-TB regimens for years to come. Despite inclusion in treatment guidelines and widespread deployment in TB programmes, the optimal use of linezolid in RR-TB is unknown. Its narrow therapeutic window, resulting from shared efficacy and toxicity targets, is a challenge for optimised dosing. The standard 600 mg dose has been inadequately studied, particularly in programmatic settings with high HIV burden, and was selected based limited empirical PK and clinical toxicity data [95]. Evidence-based linezolid dosing is needed to support continued use. Furthermore, the frequency and genetic correlates of linezolid resistance in TB programmes are poorly described, but essential to inform strategies for resistance testing and development of rapid drug susceptibility testing as use expands. This thesis presents a series of studies designed to address these knowledge gaps around linezolid use in programmatic settings.

Chapter 3 reports non-compartmental analysis describing linezolid PK among 30 South African patients with RR-TB and HIV prevalence of 50%. Consistent with other studies [99], there was large inter-individual variation in linezolid plasma concentrations, with an overall coefficient of variation (%CV) of 40.1%. Much of the observed variability was unexplained, although age and weight were identified as significant predictors of trough concentrations and $AUC_{0\text{-}24}$, respectively. Importantly, linezolid exposures were not different among HIV-positive participants. Similar values for clearance and central volume of distribution were estimated from a population PK model using the same data, which also did not find a significant association between HIV and linezolid PK exposure [251]. Data presented in Chapter 3 confirm several PK characteristics that make linezolid well-suited for TDM, a potential strategy to reduce toxicity if target thresholds

can be established: large inter-individual variability, rapid attainment of steady state, linear relationship between AUC and trough concentrations supporting limited sampling, and proportional dose-exposure relationships.

A probability of target attainment analysis was performed using observed PK exposures and MIC distributions from the study population. All participants were predicted to achieve the *in vitro* efficacy target (*f*AUC/MIC 119) at the 600 mg dose, providing indirect support for potential clinical efficacy at wild-type MICs. However, the proportion decreased to only 61.5% (95% CI, 40.6 to 79.8) at the linezolid critical concentration of 1.0 mg/L. Over half of participants had trough concentrations exceeding a toxicity threshold of 2 mg/L, illustrating the narrow therapeutic window. This target, which is not externally validated, was established among a small HIV-negative Korean cohort [83], emphasising the need for clinical evaluation of the standard 600 mg dose in other settings.

Building on these findings, **Chapter 4** presents a prospective observational cohort study to characterise clinical linezolid toxicity among 151 patients in the South African RR-TB treatment programme. A major strength of the study was integration of linezolid PK parameters in models to explore predictors of adverse events and identify a concentration threshold for clinical toxicity. Linezolid was frequently interrupted or prematurely discontinued by clinicians, reflecting widespread perception of serious toxicity and a low threshold to stop this important drug during TB therapy. Mild adverse events also occurred frequently, dominated by grade 1 anaemia and peripheral neuropathy, in keeping with reports from other settings. However, cumulative incidence of new grade 3 or 4 events was less common, with an incidence of 14% (95% CI, 9 - 21) at 6 months; there were only six grade 4 anaemia events and one grade 4 peripheral neuropathy event.

Thrombocytopenia, widely used as a measure of linezolid toxicity in Gram-positive infection and TB studies, was a poor pharmacodynamic marker because of the non-specific nature of platelet reduction from improvement of acute phase response during treatment. In contrast, decreased haemoglobin concentration is more specific for linezolid toxicity and was selected as the primary outcome measure. There was an increase in average haemoglobin over time, after adjustment for time-varying linezolid

concentrations, HIV status, age, sex, and baseline haemoglobin values. These findings suggest a positive overall treatment effect of linezolid-based antituberculosis therapy and supports use of the 600 mg dose in TB programmes with high rates of HIV co-infection. Preliminary findings from the ZeNix trial provide additional evidence for use of the standard 600 mg dose, reporting equivalent clinical efficacy and reduced adverse events compared with higher doses [283].

Factors associated with clinically significant haemoglobin reductions (> 2 g/dL) included linezolid trough concentration, male sex, and age. HIV-positivity was not a significant predictor. This may help to select patients at high risk for anaemia and develop a more targeted approach to monitoring. A trough threshold of 2.5 mg/L resulted in optimal model fit to describe change in haemoglobin and was strongly associated with risk of anaemia, after adjustment for other factors. The specificity of this cut off for anaemia events was excellent - 8 out of 9 (89%) participants with a linezolid trough concentration above 2.5 mg/L developed anaemia, defined as a drop in haemoglobin > 2 g/dL, within the first 4 months of therapy - with potential for use in therapeutic drug monitoring (TDM) to reduce risk of adverse events, either by triggering more intensive monitoring or dose reduction. This finding requires external validation and clinical evaluation as a strategy for toxicity reduction if confirmed.

Even if shown to accurately predict toxicity, it is unclear whether a TDM strategy for linezolid will lead to meaningful reductions in adverse events given the low proportion of patients with values above the 2.5 mg/L cut off (a trial to test this will require a large sample size to show an effect). It is also unknown how dose reductions with TDM will impact efficacy; only half of *f*AUC estimates for trough concentrations < 2.5 mg/L were above the putative efficacy threshold of 119 with linezolid dosing at 600 mg (assuming an MIC of 1 mg/L), but the relationship between this PK target and clinical outcomes is not established. Linezolid is stable on dried blood spots which are simple to obtain and could be centrally tested [225, 284], but limited availability of linezolid drug assays in high TB burden settings is major drawback of TDM. Semiquantitative measurements using mobile spectroscopy devices on non-invasive specimens such as saliva may facilitate programmatic implementation of TDM [285]. This approach, in combination with rapid genotypic testing as a surrogate for MIC, may be an attractive intervention to test for feasibility and effectiveness in a prospective study. Other strategies to identify

patients at high risk for adverse events could be effective. Pharmacodynamic modelling of data from the Nix-TB trial (where linezolid was dosed at 1200 mg daily) showed that a 10% reduction in haemoglobin had good accuracy for predicting severe anaemia and could be useful as a trigger for dose reduction [265].

Impact of linezolid dose reduction on resistance was part of the rationale for the study presented in **Chapter 5**, which described the clinical phenotype and genotypic correlates of linezolid resistance among 39 patients with treatment failure on linezolid-based therapy for RR-TB. Despite prolonged therapy with linezolid - median 16 months until the last culture - and weak background regimens, linezolid resistance was detected in only a third of patients and occurred late (range 7 to 27 months after initiating linezolid). Linezolid dose reduction to 300 mg was not associated with resistance. These clinical observations are in keeping with the high *in vitro* genetic barrier to resistance [113, 118] and provide reassurance for programmatic use, particularly in combination with potent new agents like bedaquiline. The other major finding was that phenotypic linezolid resistance was predicted by a limited number of mutations in two genes, *rrl* and *rplC*, most of which are previously reported in both clinical and laboratory *M. tuberculosis* isolates. Importantly, there appears to be a class effect for key linezolid resistance mutations [113, 123, 269]. This provides strong rationale for development of molecular diagnostic tests, such as line probe assays, to support programmatic deployment of linezolid and new oxazolidinones. With the inevitable rise in prevalence of population-level resistance with expanded use, screening for genotypic resistance should be considered for patients with a positive culture after 4 months of linezolid therapy.

This thesis sought to address key knowledge gaps in the use of linezolid for RR-TB, with the objective of informing treatment practice. The series of studies presented here contribute to the evidence base for continued use of the 600 mg dose in TB treatment programmes, demonstrating attainment of PK-efficacy targets in a population with high HIV prevalence, characterising the clinical toxicity profile and confirming an exposure-response relationship for toxicity, and describing genotypic correlates of linezolid resistance. Important uncertainties remain, however, including the role of 1200 mg dosing and optimal duration of linezolid therapy that optimises efficacy and minimises toxicity, and use of linezolid in extra-pulmonary forms of TB. The phase 3 ZeNix trial

(NCT03086486), mentioned above, is evaluating four different linezolid dosing strategies as part of an all-oral shorter regimen for pulmonary RR-TB. Preliminary results suggest that 600 mg dosing had similar outcomes compared with 1200 mg when combined with new antituberculosis agents, but with fewer adverse events [283]. Linezolid did not contribute improved culture conversion in a phase 2b trial for drug-susceptible TB [254] - a drug-drug interaction may have contributed to suboptimal linezolid exposure [153] - and is unlikely to be used as a component of rifampicin-based regimens in pulmonary TB.

Linezolid is an attractive agent for intensified antimicrobial therapy in TB meningitis because of excellent penetration into cerebrospinal fluid [286-288], and several trials are underway to investigate use in this setting. New oxazolidines with greater *M. tuberculosis* selectivity and that are less toxic to mammalian mitochondria are in development. Clinical development of sutezolid (PNU-100480) was delayed after showing promising results in phase 1 and 2 evaluation, but it has now entered a dose-finding study as part of novel combination therapy for drug-susceptible TB (NCT03959566). The highly-potent contezolid (MRX-4/MRX-1) [289] and TBI-223 (NCT03758612) compounds are in phase 1 development, and delpazolid [269] has entered phase 2 testing (NCT02836483). It is unknown whether the favourable preclinical characteristics of novel oxazolidinones will translate into improved safety in patients, and it will likely take several years to define the role for these agents. In the meantime, linezolid will continue to be widely prescribed as a component of RR-TB regimens. Building on the body of work in this thesis, future research priorities should include individualised risk reduction strategies for adverse events, clinical evaluation of TDM-based dosing, and development of rapid molecular diagnostic tests for linezolid resistance.

REFERENCES

1. World Health Organization. Global tuberculosis report 2021. Geneva, **2021**.
2. Lönnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010–50: cure, care, and social development. Lancet **2010**; 375(9728): 1814-29.
3. Fox W, Sutherland I, Daniels M. A five-year assessment of patients in a controlled trial of streptomycin in pulmonary tuberculosis; report to the Tuberculosis Chemotherapy Trials Committee of the Medical Research Council. Q J Med **1954**; 23(91): 347-66.
4. Mitchison D, Davies G. The chemotherapy of tuberculosis: past, present and future. Int J Tuberc Lung Dis **2012**; 16(6): 724-32.
5. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946-1986, with relevant subsequent publications. Int J Tuberc Lung Dis **1999**; 3(10 Suppl 2): S231-79.
6. East African/British Medical Research Council. Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet **1972**; 1(7760): 1079-85.
7. Pouplin T, Bang ND, Toi PV, et al. Naive-pooled pharmacokinetic analysis of pyrazinamide, isoniazid and rifampicin in plasma and cerebrospinal fluid of Vietnamese children with tuberculous meningitis. BMC Infect Dis **2016**; 16: 144.
8. Aarnoutse RE, Kibiki GS, Reither K, et al. Pharmacokinetics, Tolerability, and Bacteriological Response of Rifampin Administered at 600, 900, and 1,200 Milligrams Daily in Patients with Pulmonary Tuberculosis. Antimicrob Agents Chemother **2017**; 61(11): e01054-17.
9. Angeby K, Jureen P, Kahlmeter G, Hoffner SE, Schon T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for Mycobacterium tuberculosis. Bull World Health Organ **2012**; 90(9): 693-8.
10. Naidoo A, Chirehwa M, McIlleron H, et al. Effect of rifampicin and efavirenz on moxifloxacin concentrations when co-administered in patients with drug-susceptible TB. J Antimicrob Chemother **2017**; 72(5): 1441-9.
11. Lipsitch M, Levin BR. Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. Int J Tuberc Lung Dis **1998**; 2(3): 187-99.
12. Gillespie SH. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrob Agents Chemother **2002**; 46(2): 267-74.
13. Yang C, Luo T, Shen X, et al. Transmission of multidrug-resistant Mycobacterium tuberculosis in Shanghai, China: a retrospective observational study using whole-genome sequencing and epidemiological investigation. Lancet Infect Dis **2017**; 17(3): 275-84.
14. Sharma A, Hill A, Kurbatova E, et al. Estimating the future burden of multidrug-resistant and extensively drug-resistant tuberculosis in India, the Philippines, Russia, and South Africa: a mathematical modelling study. Lancet Infect Dis **2017**; 17(7): 707-15.
15. Fitzpatrick C, Floyd K. A systematic review of the cost and cost effectiveness of treatment for multidrug-resistant tuberculosis. PharmacoEconomics **2012**; 30(1): 63-80.
16. Falzon D, Gandhi N, Migliori GB, et al. Resistance to fluoroquinolones and second-line injectable drugs: impact on multidrug-resistant TB outcomes. Eur Respir J **2013**; 42(1): 156-68.
17. Jacobson K, Barnard M, Buckley M, et al. A rifampicin-resistant diagnosis on Xpert MTB/RIF frequently leads to initiation of a weakened standardized MDR-TB regimen. 46th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease. Cape Town, **2015**.
18. Wu S, Zhang Y, Sun F, et al. Adverse Events Associated With the Treatment of Multidrug-Resistant Tuberculosis: A Systematic Review and Meta-analysis. Am J Ther **2016**; 23(2): e521-30.
19. Bastos ML, Lan Z, Menzies D. An updated systematic review and meta-analysis for treatment of multidrug-resistant tuberculosis. Eur Respir J **2017**; 49(3).
20. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect Dis **2009**; 9(3): 153-61.
21. Floyd K, Hutubessy R, Kliiman K, et al. Cost and cost-effectiveness of multidrug-resistant tuberculosis treatment in Estonia and Russia. Eur Respir J **2012**; 40(1): 133-42.
22. Diel R, Nienhaus A, Lampenius N, Rusch-Gerdes S, Richter E. Cost of multi drug resistance tuberculosis in Germany. Respir Med **2014**; 108(11): 1677-87.
23. World Health Organization. Global tuberculosis report 2018. Geneva, Switzerland, **2018**.
24. World Health Organization. Global tuberculosis report 2017. Geneva, Switzerland, **2017**.
25. World Health Organization. Global tuberculosis report 2016. Geneva, Switzerland, **2016**.

26. World Health Organization. Global Tuberculosis Report 2015. Geneva, Switzerland, **2015**.
27. Bastard M, Sanchez-Padilla E, du Cros P, et al. Outcomes of HIV-infected versus HIV-non-infected patients treated for drug-resistance tuberculosis: Multicenter cohort study. PloS one **2018**; 13(3): e0193491.
28. Alemu A, Bitew ZW, Worku T, Gamtesa DF, Alebel A. Predictors of mortality in patients with drug-resistant tuberculosis: A systematic review and meta-analysis. PloS one **2021**; 16(6): e0253848.
29. World Health Organization. Global tuberculosis report 2020. Geneva, Switzerland, **2020**.
30. Pietersen E, Ignatius E, Streicher EM, et al. Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study. Lancet **2014**; 383(9924): 1230-9.
31. Mphahlele M, Warren RM, Streicher E, Van Der Walt M, Van Helden P, Jacobson K. Emergence of additional resistance during standardized MDR-TB treatment. 46th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease Cape Town, **2015**.
32. Muller B, Chihota VN, Pillay M, et al. Programmatically selected multidrug-resistant strains drive the emergence of extensively drug-resistant tuberculosis in South Africa. PloS one **2013**; 8(8): e70919.
33. Zumla A, Hafner R, Lienhardt C, Hoelscher M, Nunn A. Advancing the development of tuberculosis therapy. Nat Rev Drug Discov **2012**; 11(3): 171-2.
34. Iseman MD. Treatment of multidrug-resistant tuberculosis. N Engl J Med **1993**; 329(11): 784-91.
35. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-analysis. PloS one **2009**; 4(9): e6914.
36. Ahuja SD, Ashkin D, Avendano M, et al. Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. PLoS med **2012**; 9(8): e1001300.
37. Fox GJ, Benedetti A, Cox H, et al. Group 5 drugs for multidrug-resistant tuberculosis: individual patient data meta-analysis. Eur Respir J **2017**; 49(1).
38. Ahmad N, Ahuja SD, Akkerman OW, et al. Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet **2018**; 392(10150): 821-34.
39. Trebucq A, Schwoebel V, Kashongwe Z, et al. Treatment outcome with a short multidrug-resistant tuberculosis regimen in nine African countries. Int J Tuberc Lung Dis **2018**; 22(1): 17-25.
40. Ahmad Khan F, Salim MAH, du Cros P, et al. Effectiveness and safety of standardised shorter regimens for multidrug-resistant tuberculosis: individual patient data and aggregate data meta-analyses. Eur Respir J **2017**; 50(1).
41. Conradie F, Diacon AH, Ngubane N, et al. Treatment of Highly Drug-Resistant Pulmonary Tuberculosis. N Engl J Med **2020**; 382(10): 893-902.
42. Dorman SE, Nahid P, Kurbatova EV, et al. Four-Month Rifapentine Regimens with or without Moxifloxacin for Tuberculosis. N Engl J Med **2021**; 384(18): 1705-18.
43. Van Deun A, Maug AK, Salim MA, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med **2010**; 182(5): 684-92.
44. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis 2016 update. Geneva, **2016**.
45. Aung KJ, Van Deun A, Declercq E, et al. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis **2014**; 18(10): 1180-7.
46. Kuaban C, Noeske J, Rieder HL, Ait-Khaled N, Abena Foe JL, Trebucq A. High effectiveness of a 12-month regimen for MDR-TB patients in Cameroon. Int J Tuberc Lung Dis **2015**; 19(5): 517-24.
47. Piubello A, Harouna SH, Souleymane MB, et al. High cure rate with standardised short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. Int J Tuberc Lung Dis **2014**; 18(10): 1188-94.
48. Nunn AJ, Phillips PPJ, Meredith SK, et al. A Trial of a Shorter Regimen for Rifampin-Resistant Tuberculosis. N Engl J Med **2019**; 380(13): 1201-13.
49. Tyagi S, Ammerman NC, Li SY, et al. Clofazimine shortens the duration of the first-line treatment regimen for experimental chemotherapy of tuberculosis. Proc Natl Acad Sci U.S.A **2015**; 112(3): 869-74.
50. Grosset JH, Tyagi S, Almeida DV, et al. Assessment of clofazimine activity in a second-line regimen for tuberculosis in mice. Am J Respir Crit Care Med **2013**; 188(5): 608-12.
51. Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science **2005**; 307(5707): 223-7.
52. Koul A, Dendouga N, Vergauwen K, et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nat Chem Biol **2007**; 3(6): 323-4.

53. Veziris N, Ibrahim M, Lounis N, Andries K, Jarlier V. Sterilizing activity of second-line regimens containing TMC207 in a murine model of tuberculosis. PloS one **2011**; 6(3): e17556.
54. Williams K, Minkowski A, Amoabeng O, et al. Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother **2012**; 56(6): 3114-20.
55. Tasneen R, Li SY, Peloquin CA, et al. Sterilizing activity of novel TMC207- and PA-824-containing regimens in a murine model of tuberculosis. Antimicrob Agents Chemother **2011**; 55(12): 5485-92.
56. Koul A, Vranckx L, Dendouga N, et al. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. The Journal of biological chemistry **2008**; 283(37): 25273-80.
57. Diacon AH, Pym A, Grobusch MP, et al. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. N Engl J Med **2014**; 371(8): 723-32.
58. Pym AS, Diacon AH, Tang SJ, et al. Bedaquiline in the treatment of multidrug- and extensively drug-resistant tuberculosis. Eur Respir J **2016**; 47(2): 564-74.
59. Zhao Y, Fox T, Manning K, et al. Improved Treatment Outcomes With Bedaquiline When Substituted for Second-line Injectable Agents in Multidrug-resistant Tuberculosis: A Retrospective Cohort Study. Clin Infect Dis **2018**; 68(9): 1522-9.
60. Schnippel K, Ndjeka N, Maartens G, et al. Effect of bedaquiline on mortality in South African patients with drug-resistant tuberculosis: a retrospective cohort study. Lancet Respir Med **2018**; 6(9): 699-706.
61. World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. Geneva, **2019**.
62. Mirzayev F, Viney K, Linh NN, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. Eur Respir J **2020**; 57(6):2003300.
63. Lee M, Lee J, Carroll MW, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med **2012**; 367(16): 1508-18.
64. Lee M, Cho SN, Barry CE, 3rd, Song T, Kim Y, Jeong I. Linezolid for XDR-TB--Final Study Outcomes. N Engl J Med **2015**; 373(3): 290-1.
65. Bozdogan B, Appelbaum PC. Oxazolidinones: activity, mode of action, and mechanism of resistance. Int J Antimicrob Agents **2004**; 23(2): 113-9.
66. Diekema DJ, Jones RN. Oxazolidinone antibiotics. Lancet **2001**; 358(9297): 1975-82.
67. Alcala L, Ruiz-Serrano MJ, Perez-Fernandez Turegano C, et al. In Vitro Activities of Linezolid against Clinical Isolates of Mycobacterium tuberculosis That Are Susceptible or Resistant to First-Line Antituberculous Drugs. Antimicrob Agents Chemother **2003**; 47(1): 416-7.
68. Rodriguez JC, Ruiz M, Lopez M, Royo G. In vitro activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against Mycobacterium tuberculosis. Int J Antimicrob Agents **2002**; 20(6): 464-7.
69. Zurenko GE, Yagi BH, Schaadt RD, et al. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob Agents Chemother **1996**; 40(4): 839-45.
70. Cynamon MH, Klemens SP, Sharpe CA, Chase S. Activities of several novel oxazolidinones against Mycobacterium tuberculosis in a murine model. Antimicrob Agents Chemother **1999**; 43(5): 1189-91.
71. Ashtekar DR, Costa-Periera R, Shrinivasan T, Iyyer R, Vishvanathan N, Rittel W. Oxazolidinones, a new class of synthetic antituberculosis agent. In vitro and in vivo activities of DuP-721 against Mycobacterium tuberculosis. Diagn Microbiol Infect Dis **1991**; 14(6): 465-71.
72. Williams KN, Stover CK, Zhu T, et al. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. Antimicrob Agents Chemother **2009**; 53(4): 1314-9.
73. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. Am J Respir Crit Care Med **2008**; 178(11): 1180-5.
74. Diacon AH, De Jager VR, Dawson R, et al. Fourteen-Day Bactericidal Activity, Safety, and Pharmacokinetics of Linezolid in Adults with Drug-Sensitive Pulmonary Tuberculosis. Antimicrob Agents Chemother **2020**; 64(4):e02012-19.
75. Franke MF, Khan P, Hewison C, et al. Culture Conversion in Patients Treated with Bedaquiline and/or Delamanid: A Prospective Multi-country Study. Am J Respir Crit Care Med **2021**; 203(1):111-119.
76. Skrahina A. Bedaquiline containing regimens in the treatment of multi- and extensively drug-resistant tuberculosis at the programmatic level: prospective cohort study. 49th Union World Conference on Lung Health. The Hague, the Netherlands., **2018**.
77. De Vriese AS, Coster RV, Smet J, et al. Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis **2006**; 42(8): 1111-7.

78. Pacheu-Grau D, Gomez-Duran A, Lopez-Perez MJ, Montoya J, Ruiz-Pesini E. Mitochondrial pharmacogenomics: barcode for antibiotic therapy. Drug Discov Today **2010**; 15(1-2): 33-9.
79. Beekmann SE, Gilbert DN, Polgreen PM, Network IEI. Toxicity of extended courses of linezolid: results of an Infectious Diseases Society of America Emerging Infections Network survey. Diagn Microbiol Infect Dis **2008**; 62(4): 407-10.
80. McDowell A, Haas M, Seaworth B, et al. Linezolid use for the treatment of multidrug-resistant tuberculosis, TB centers of excellence, United States, 2013-2018. J Clin Tuberc Other Mycobact Dis **2021**; 22: 100201.
81. Zhang X, Falagas ME, Vardakas KZ, et al. Systematic review and meta-analysis of the efficacy and safety of therapy with linezolid containing regimens in the treatment of multidrug-resistant and extensively drug-resistant tuberculosis. J Thorac Dis **2015**; 7(4): 603-15.
82. Palenzuela L, Hahn NM, Nelson RP, Jr., et al. Does linezolid cause lactic acidosis by inhibiting mitochondrial protein synthesis? Clin Infect Dis **2005**; 40(12): e113-6.
83. Song T, Lee M, Jeon H-S, et al. Linezolid Trough Concentrations Correlate with Mitochondrial Toxicity-Related Adverse Events in the Treatment of Chronic Extensively Drug-Resistant Tuberculosis. EBioMedicine **2015**; 2(11): 1627-33.
84. Centner CM, Bateman KJ, Heckmann JM. Manifestations of HIV infection in the peripheral nervous system. Lancet Neurol **2013**; 12(3): 295-309.
85. Mateu de Antonio J, Grau S, Morales-Molina JA, Marin-Casino M. Thrombocytopenia and anemia associated with linezolid in patients with kidney failure. Clin Infect Dis **2006**; 42(10): 1500; author reply 1.
86. Lin YH, Wu VC, Tsai IJ, et al. High frequency of linezolid-associated thrombocytopenia among patients with renal insufficiency. Int J Antimicrob Agents **2006**; 28(4): 345-51.
87. Matsumoto K, Takeshita A, Ikawa K, et al. Higher linezolid exposure and higher frequency of thrombocytopenia in patients with renal dysfunction. Int J Antimicrob Agents **2010**; 36(2): 179-81.
88. Nukui Y, Hatakeyama S, Okamoto K, et al. High plasma linezolid concentration and impaired renal function affect development of linezolid-induced thrombocytopenia. J Antimicrob Chemother **2013**; 68(9): 2128-33.
89. Sasaki T, Takane H, Ogawa K, et al. Population pharmacokinetic and pharmacodynamic analysis of linezolid and a hematologic side effect, thrombocytopenia, in Japanese patients. Antimicrob Agents Chemother **2011**; 55(5): 1867-73.
90. McKee EE, Ferguson M, Bentley AT, Marks TA. Inhibition of mammalian mitochondrial protein synthesis by oxazolidinones. Antimicrob Agents Chemother **2006**; 50(6): 2042-9.
91. Koh WJ, Kwon OJ, Gwak H, et al. Daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. J Antimicrob Chemother **2009**; 64(2): 388-91.
92. Plock N, Buerger C, Joukhadar C, Kljucar S, Kloft C. Does linezolid inhibit its own metabolism? Population pharmacokinetics as a tool to explain the observed nonlinearity in both healthy volunteers and septic patients. Drug Metab Dispos **2007**; 35(10): 1816-23.
93. Keel RA, Schaeftlein A, Kloft C, et al. Pharmacokinetics of intravenous and oral linezolid in adults with cystic fibrosis. Antimicrob Agents Chemother **2011**; 55(7): 3393-8.
94. Boak LM, Rayner CR, Grayson ML, et al. Clinical population pharmacokinetics and toxicodynamics of linezolid. Antimicrob Agents Chemother **2014**; 58(4): 2334-43.
95. Bolhuis MS, Akkerman OW, Sturkenboom MGG, et al. Linezolid-based Regimens for Multidrug-resistant Tuberculosis (TB): A Systematic Review to Establish or Revise the Current Recommended Dose for TB Treatment. Clin Infect Dis **2018**; 67(suppl_3): S327-s35.
96. McIlleron H MG, Burman WJ, Maartens G. Complications of Antiretroviral Therapy in Patients with Tuberculosis: Drug Interactions, Toxicity, and Immune Reconstitution Inflammatory Syndrome. J Infect Dis **2007**; 196: S63–75.
97. McIlleron H, Rustomjee R, Vahedi M, et al. Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: implications for international dosing guidelines. Antimicrob Agents Chemother **2012**; 56(6): 3232-8.
98. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob Agents Chemother **2006**; 50(4): 1170-7.
99. Millard J, Pertinez H, Bonnett L, et al. Linezolid pharmacokinetics in MDR-TB: a systematic review, meta-analysis and Monte Carlo simulation. J Antimicrob Chemother **2018**; 73(7):1755-1762.

100. Pea F, Furlanut M, Cojutti P, et al. Therapeutic drug monitoring of linezolid: a retrospective monocentric analysis. Antimicrob Agents Chemother **2010**; 54(11): 4605-10.
101. Yagi T, Naito T, Doi M, et al. Plasma exposure of free linezolid and its ratio to minimum inhibitory concentration varies in critically ill patients. Int J Antimicrob Agents **2013**; 42(4): 329-34.
102. Meagher AK, Forrest A, Rayner CR, Birmingham MC, Schentag JJ. Population pharmacokinetics of linezolid in patients treated in a compassionate-use program. Antimicrob Agents Chemother **2003**; 47(2): 548-53.
103. Abe S, Chiba K, Cirincione B, Grasela TH, Ito K, Suwa T. Population pharmacokinetic analysis of linezolid in patients with infectious disease: application to lower body weight and elderly patients. J Clin Pharmacol **2009**; 49(9): 1071-8.
104. Cojutti P, Pai MP, Pea F. Population Pharmacokinetics and Dosing Considerations for the Use of Linezolid in Overweight and Obese Adult Patients. Clin Pharmacokinet **2018**; 57(8): 989-1000.
105. Brown AN, Drusano GL, Adams JR, et al. Preclinical Evaluations To Identify Optimal Linezolid Regimens for Tuberculosis Therapy. mBio **2015**; 6(6): e01741-15.
106. Drusano GL, Neely M, Van Guilder M, et al. Analysis of combination drug therapy to develop regimens with shortened duration of treatment for tuberculosis. PloS one **2014**; 9(7): e101311.
107. Srivastava S, Magombedze G, Koeuth T, et al. Linezolid Dose That Maximizes Sterilizing Effect While Minimizing Toxicity and Resistance Emergence for Tuberculosis. Antimicrob Agents Chemother **2017**; 61(8):e00751-17.
108. Cazavet J, Bounes FV, Ruiz S, et al. Risk factor analysis for linezolid-associated thrombocytopenia in critically ill patients. Eur J Clin Microbiol Infect Dis **2020**; 39(3): 527-38.
109. Pea F, Viale P, Cojutti P, Del Pin B, Zamparini E, Furlanut M. Therapeutic drug monitoring may improve safety outcomes of long-term treatment with linezolid in adult patients. J Antimicrob Chemother **2012**; 67(8): 2034-42.
110. Sotgiu G, Centis R, D'Ambrosio L, et al. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. Eur Respir J **2012**; 40(6): 1430-42.
111. Richter E, Rusch-Gerdes S, Hillemann D. First linezolid-resistant clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother **2007**; 51(4): 1534-6.
112. Du J, Gao J, Yu Y, et al. Low Rate of Acquired Linezolid Resistance in Multidrug-Resistant Tuberculosis Treated With Bedaquiline-Linezolid Combination. Front Microbiol **2021**; 12: 655653.
113. McNeil MB, Dennison D, Shelton C, Parish T. In vitro isolation and characterization of oxazolidinone resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother **2017**; 61(10):e01296-17.
114. Zimenkov DV, Nosova EY, Kulagina EV, et al. Examination of bedaquiline- and linezolid-resistant Mycobacterium tuberculosis isolates from the Moscow region. J Antimicrob Chemother **2017**; 72(7): 1901-6.
115. Bloemberg GV, Keller PM, Stucki D, et al. Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. N Engl J Med **2015**; 373(20): 1986-8.
116. Somoskovi A, Bruderer V, Homke R, Bloemberg GV, Bottger EC. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. Eur Respir J **2015**; 45(2): 554-7.
117. Hillemann D, Rusch-Gerdes S, Richter E. In vitro-selected linezolid-resistant Mycobacterium tuberculosis mutants. Antimicrob Agents Chemother **2008**; 52(2): 800-1.
118. Ismail N, Omar SV, Ismail NA, Peters RPH. In vitro approaches for generation of Mycobacterium tuberculosis mutants resistant to bedaquiline, clofazimine or linezolid and identification of associated genetic variants. J Microbiol Methods **2018**; 153: 1-9.
119. Zhang S, Chen J, Cui P, et al. Mycobacterium tuberculosis Mutations Associated with Reduced Susceptibility to Linezolid. Antimicrob Agents Chemother **2016**; 60(4): 2542-4.
120. Zong Z, Jing W, Shi J, et al. Comparison of In Vitro Activity and MIC Distributions between the Novel Oxazolidinone Delpazolid and Linezolid against Multidrug-Resistant and Extensively Drug-Resistant Mycobacterium tuberculosis in China. Antimicrob Agents Chemother **2018**; 62(8):e00165-18.
121. Zhang Z, Pang Y, Wang Y, Liu C, Zhao Y. Beijing genotype of Mycobacterium tuberculosis is significantly associated with linezolid resistance in multidrug-resistant and extensively drug-resistant tuberculosis in China. Int J Antimicrob Agents **2014**; 43(3): 231-5.
122. Beckert P, Hillemann D, Kohl TA, et al. rplC T460C identified as a dominant mutation in linezolid-resistant Mycobacterium tuberculosis strains. Antimicrob Agents Chemother **2012**; 56(5): 2743-5.

123. Balasubramanian V, Solapure S, Iyer H, et al. Bactericidal activity and mechanism of action of AZD5847, a novel oxazolidinone for treatment of tuberculosis. Antimicrob Agents Chemother **2014**; 58(1): 495-502.
124. Makafe GG, Cao Y, Tan Y, et al. Role of the Cys154Arg Substitution in Ribosomal Protein L3 in Oxazolidinone Resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother **2016**; 60(5): 3202-6.
125. Pang Y, Zong Z, Huo F, et al. In Vitro Drug Susceptibility of Bedaquiline, Delamanid, Linezolid, Clofazimine, Moxifloxacin, and Gatifloxacin against Extensively Drug-Resistant Tuberculosis in Beijing, China. Antimicrob Agents Chemother **2017**; 61(10):e00900-17.
126. Perdigao J, Maltez F, Machado D, et al. Beyond extensively drug-resistant tuberculosis in Lisbon, Portugal: a case of linezolid resistance acquisition presenting as an iliopsoas abscess. Int J Antimicrob Agents **2016**; 48(5): 569-70.
127. Esmail A, Oelofse S, Lombard C, et al. An All-Oral 6-Month Regimen for Multidrug-Resistant TB (the NExT Study): A Multicenter, Randomized Controlled Trial. Am J Respir Crit Care Med **2022**; 205(10):1214-1227.
128. Brust JCM, Gandhi NR, Wasserman S, et al. Effectiveness and cardiac safety of bedaquiline-based therapy for drug-resistant tuberculosis: a prospective cohort study. Clin Infect Dis **2021**; 73(11):2083-2092.
129. McArthur JH. The reliability and validity of the subjective peripheral neuropathy screen. J Assoc Nurses AIDS Care **1998**; 9(4): 84-94.
130. Springer B, Lucke K, Calligaris-Maibach R, Ritter C, Bottger EC. Quantitative drug susceptibility testing of Mycobacterium tuberculosis by use of MGIT 960 and EpiCenter instrumentation. J Clin Microbiol **2009**; 47(6): 1773-80.
131. Martin A, Portaels F, Palomino JC. Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis. J Antimicrob Chemother **2007**; 59(2): 175-83.
132. Nathanson E, Gupta R, Huamani P, et al. Adverse events in the treatment of multidrug-resistant tuberculosis: results from the DOTS-Plus initiative. Int J Tuberc Lung Dis **2004**; 8(11): 1382-4.
133. Migliori GB, Eker B, Richardson MD, et al. A retrospective TBNET assessment of linezolid safety, tolerability and efficacy in multidrug-resistant tuberculosis. Eur Respir J **2009**; 34(2): 387-93.
134. Zhou CC, Swaney SM, Shinabarger DL, Stockman BJ. 1H nuclear magnetic resonance study of oxazolidinone binding to bacterial ribosomes. Antimicrob Agents Chemother **2002**; 46(3): 625-9.
135. Leach KL, Swaney SM, Colca JR, et al. The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. Molecular cell **2007**; 26(3): 393-402.
136. Swaney SM, Aoki H, Ganoza MC, Shinabarger DL. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob Agents Chemother **1998**; 42(12): 3251-5.
137. Livermore DM. Linezolid in vitro: mechanism and antibacterial spectrum. J Antimicrob Chemother **2003**; 51 Suppl 2: ii9-16.
138. Lin AH, Murray RW, Vidmar TJ, Marotti KR. The oxazolidinone eperezolid binds to the 50S ribosomal subunit and competes with binding of chloramphenicol and lincomycin. Antimicrob Agents Chemother **1997**; 41(10): 2127-31.
139. Fines M, Leclercq R. Activity of linezolid against Gram-positive cocci possessing genes conferring resistance to protein synthesis inhibitors. J Antimicrob Chemother **2000**; 45(6): 797-802.
140. Dryden MS. Linezolid pharmacokinetics and pharmacodynamics in clinical treatment. J Antimicrob Chemother **2011**; 66 Suppl 4: iv7-iv15.
141. Di Paolo A, Malacarne P, Guidotti E, Danesi R, Del Tacca M. Pharmacological issues of linezolid: an updated critical review. Clin Pharmacokinet **2010**; 49(7): 439-47.
142. Tan TQ, Yogev R. Clinical pharmacology of linezolid: an oxazolidinone antimicrobial agent. Expert Rev Clin Pharmacol **2008**; 1(4): 479-89.
143. Gee T, Ellis R, Marshall G, Andrews J, Ashby J, Wise R. Pharmacokinetics and tissue penetration of linezolid following multiple oral doses. Antimicrob Agents Chemother **2001**; 45(6): 1843-6.
144. Dehghanyar P, Burger C, Zeitlinger M, et al. Penetration of linezolid into soft tissues of healthy volunteers after single and multiple doses. Antimicrob Agents Chemother **2005**; 49(6): 2367-71.
145. Tsona A, Metallidis S, Foroglou N, et al. Linezolid penetration into cerebrospinal fluid and brain tissue. J Chemother **2010**; 22(1): 17-9.
146. Akkerman OW, van Altena R, Klinkenberg T, et al. Drug concentration in lung tissue in multidrug-resistant tuberculosis. Eur Respir J **2013**; 42(6): 1750-2.

147. Conte JE, Golden JA, Kipps J, Zurlinden E. Intrapulmonary Pharmacokinetics of Linezolid. Antimicrob Agents Chemother **2002**; 46(5): 1475-80.
148. Honeybourne D, Tobin C, Jevons G, Andrews J, Wise R. Intrapulmonary penetration of linezolid. J Antimicrob Chemother **2003**; 51(6): 1431-4.
149. Chang KC, Yew WW, Cheung SW, et al. Can intermittent dosing optimize prolonged linezolid treatment of difficult multidrug-resistant tuberculosis? Antimicrob Agents Chemother **2013**; 57(7): 3445-9.
150. Stevens DL, Dotter B, Madaras-Kelly K. A review of linezolid: the first oxazolidinone antibiotic. Expert Rev Anti Infect Ther **2004**; 2(1): 51-9.
151. Slatter JG, Stalker DJ, Feenstra KL, et al. Pharmacokinetics, metabolism, and excretion of linezolid following an oral dose of [(14)C]linezolid to healthy human subjects. Drug Metab Dispos **2001**; 29(8): 1136-45.
152. Han T, Naicker S, Ramdial P, Assounga A. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. Kidney Int **2006**; 69(12): 2243-50.
153. Gandelman K, Zhu T, Fahmi OA, et al. Unexpected effect of rifampin on the pharmacokinetics of linezolid: in silico and in vitro approaches to explain its mechanism. J Clin Pharmacol **2011**; 51(2): 229-36.
154. Bolhuis MS, van Altena R, van Soolingen D, et al. Clarithromycin increases linezolid exposure in multidrug-resistant tuberculosis patients. Eur Respir J **2013**; 42(6): 1614-21.
155. Gebhart BC, Barker BC, Markewitz BA. Decreased serum linezolid levels in a critically ill patient receiving concomitant linezolid and rifampin. Pharmacotherapy **2007**; 27(3): 476-9.
156. Bolhuis MS, van Altena R, Uges DR, van der Werf TS, Kosterink JG, Alffenaar JW. Clarithromycin significantly increases linezolid serum concentrations. Antimicrob Agents Chemother **2010**; 54(12): 5418-9.
157. Gervasoni C, Simonetti FR, Resnati C, Charbe N, Clementi E, Cattaneo D. Prolonged inductive effect of rifampicin on linezolid exposure. European journal of clinical pharmacology **2015**; 71(5): 643-4.
158. Lawrence KR, Adra M, Gillman PK. Serotonin toxicity associated with the use of linezolid: a review of postmarketing data. Clin Infect Dis **2006**; 42(11): 1578-83.
159. Packer S, Berman SA. Serotonin syndrome precipitated by the monoamine oxidase inhibitor linezolid. Am J Psychiatry **2007**; 164(2): 346-7.
160. Go AC, Golightly LK, Barber GR, Barron MA. Linezolid interaction with serotonin reuptake inhibitors: report of two cases and incidence assessment. Drug Metab Drug Interact **2010**; 25(1-4): 41-7.
161. Taylor JJ, Wilson JW, Estes LL. Linezolid and serotonergic drug interactions: a retrospective survey. Clin Infect Dis **2006**; 43(2): 180-7.
162. Zyvoxid package insert. Available at: http://labeling.pfizer.com/ShowLabeling.aspx?id=1045. Accessed Accessed 1 June 2016.
163. Rey-Jurado E, Tudo G, Soy D, Gonzalez-Martin J. Activity and interactions of levofloxacin, linezolid, ethambutol and amikacin in three-drug combinations against Mycobacterium tuberculosis isolates in a human macrophage model. Int J Antimicrob Agents **2013**; 42(6): 524-30.
164. Rey-Jurado E, Tudo G, de la Bellacasa JP, Espasa M, Gonzalez-Martin J. In vitro effect of three-drug combinations of antituberculous agents against multidrug-resistant Mycobacterium tuberculosis isolates. Int J Antimicrob Agents **2013**; 41(3): 278-80.
165. Yip PC, Kam KM, Lam ET, Chan RC, Yew WW. In vitro activities of PNU-100480 and linezolid against drug-susceptible and drug-resistant Mycobacterium tuberculosis isolates. Int J Antimicrob Agents **2013**; 42(1): 96-7.
166. Weiss T, Schonfeld N, Otto-Knapp R, et al. Low minimal inhibitory concentrations of linezolid against multidrug-resistant tuberculosis strains. Eur Respir J **2015**; 45(1): 285-7.
167. Schon T, Jureen P, Chryssanthou E, et al. Wild-type distributions of seven oral second-line drugs against Mycobacterium tuberculosis. Int J Tuberc Lung Dis **2011**; 15(4): 502-9.
168. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Available at: http://mic.eucast.org/Eucast2/regShow.jsp?Id=37679. Accessed 20 May 2016.
169. Cremades R, Rodriguez JC, Garcia-Pachon E, et al. Interaction between linezolid and Mycobacterium tuberculosis in an experimental in vitro model. APMIS **2011**; 119(4-5): 304-8.
170. Ndjeka N, Conradie F, Schnippel K, et al. Treatment of drug-resistant tuberculosis with bedaquiline in a high HIV prevalence setting: an interim cohort analysis. Int J Tuberc Lung Dis **2015**; 19(8): 979-85.
171. Zhang M, Sala C, Dhar N, et al. In vitro and in vivo activities of three oxazolidinones against nonreplicating Mycobacterium tuberculosis. Antimicrob Agents Chemother **2014**; 58(6): 3217-23.

172. Zhao W, Guo Z, Zheng M, et al. Activity of linezolid-containing regimens against multidrug-resistant tuberculosis in mice. Int J Antimicrob Agents **2014**; 43(2): 148-53.
173. Tasneen R, Betoudji F, Tyagi S, et al. Contribution of Oxazolidinones to the Efficacy of Novel Regimens Containing Bedaquiline and Pretomanid in a Mouse Model of Tuberculosis. Antimicrob Agents Chemother **2016**; 60(1): 270-7.
174. Tang S, Yao L, Hao X, et al. Efficacy, safety and tolerability of linezolid for the treatment of XDR-TB: a study in China. Eur Respir J **2015**; 45(1): 161-70.
175. Hughes J, Isaakidis P, Andries A, et al. Linezolid for multidrug-resistant tuberculosis in HIV-infected and -uninfected patients. Eur Respir J **2015**; 46(1): 271-4.
176. Mohr E, Cox H, Wilkinson L, et al. Encouraging early outcomes for individualized treatment of multidrug-resistant tuberculosis using bedaquiline and linezolid in Khayelitsha. 46th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease Cape Town, **2015**.
177. Tsuji Y, Yukawa E, Hiraki Y, et al. Population pharmacokinetic analysis of linezolid in low body weight patients with renal dysfunction. J Clin Pharmacol **2013**; 53(9): 967-73.
178. Gray MW, Burger G, Lang BF. The origin and early evolution of mitochondria. Genome Biol **2001**; 2(6): Reviews1018.
179. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T. Mitochondrial threshold effects. The Biochem J **2003**; 370(Pt 3): 751-62.
180. Xiong L, Kloss P, Douthwaite S, et al. Oxazolidinone resistance mutations in 23S rRNA of Escherichia coli reveal the central region of domain V as the primary site of drug action. J Bacteriol **2000**; 182(19): 5325-31.
181. Saraste M. Oxidative phosphorylation at the fin de siecle. Science **1999**; 283(5407): 1488-93.
182. Sharma MR, Koc EC, Datta PP, Booth TM, Spremulli LL, Agrawal RK. Structure of the mammalian mitochondrial ribosome reveals an expanded functional role for its component proteins. Cell **2003**; 115(1): 97-108.
183. Soriano A, Miro O, Mensa J. Mitochondrial toxicity associated with linezolid. N Engl J Med **2005**; 353(21): 2305-6.
184. Garrabou G, Soriano A, Lopez S, et al. Reversible inhibition of mitochondrial protein synthesis during linezolid-related hyperlactatemia. Antimicrob Agents Chemother **2007**; 51(3): 962-7.
185. Nagiec EE, Wu L, Swaney SM, et al. Oxazolidinones inhibit cellular proliferation via inhibition of mitochondrial protein synthesis. Antimicrob Agents Chemother **2005**; 49(9): 3896-902.
186. Barnhill AE, Brewer MT, Carlson SA. Adverse effects of antimicrobials via predictable or idiosyncratic inhibition of host mitochondrial components. Antimicrob Agents Chemother **2012**; 56(8): 4046-51.
187. Del Pozo JL, Fernandez-Ros N, Saez E, Herrero JI, Yuste JR, Banales JM. Linezolid-induced lactic acidosis in two liver transplant patients with the mitochondrial DNA A2706G polymorphism. Antimicrob Agents Chemother **2014**; 58(7): 4227-9.
188. Mehta AB, Vulliamy T, Gordon-Smith EC, Luzzatto L. A new genetic polymorphism in the 16S ribosomal RNA gene of human mitochondrial DNA. Ann Hum Genet **1989**; 53(Pt 4): 303-10.
189. Boutoille D, Grossi O, Depatureaux A, Tattevin P. Fatal lactic acidosis after prolonged linezolid exposure for treatment of multidrug-resistant tuberculosis. Eur J Intern Med **2009**; 20(6): e134-5.
190. Bressler AM, Zimmer SM, Gilmore JL, Somani J. Peripheral neuropathy associated with prolonged use of linezolid. Lancet Infect Dis **2004**; 4(8): 528-31.
191. Narita M, Tsuji BT, Yu VL. Linezolid-associated peripheral and optic neuropathy, lactic acidosis, and serotonin syndrome. Pharmacotherapy **2007**; 27(8): 1189-97.
192. Bolhuis MS, Tiberi S, Sotgiu G, et al. Linezolid tolerability in multidrug-resistant tuberculosis: a retrospective study. Eur Respir J **2015**; 46(4): 1205-7.
193. Brinkman K, ter Hofstede HJ, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS **1998**; 12(14): 1735-44.
194. Jacotot E, Ravagnan L, Loeffler M, et al. The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. J Exp Med **2000**; 191(1): 33-46.
195. Flamm RK, Mendes RE, Hogan PA, Streit JM, Ross JE, Jones RN. Linezolid Surveillance Results for the United States (LEADER Surveillance Program 2014). Antimicrob Agents Chemother **2016**; 60(4): 2273-80.
196. Meka VG, Gold HS. Antimicrobial resistance to linezolid. Clin Infect Dis **2004**; 39(7): 1010-5.
197. Long KS, Vester B. Resistance to linezolid caused by modifications at its binding site on the ribosome. Antimicrob Agents Chemother **2012**; 56(2): 603-12.

198. Sander P, Belova L, Kidan YG, Pfister P, Mankin AS, Bottger EC. Ribosomal and non-ribosomal resistance to oxazolidinones: species-specific idiosyncrasy of ribosomal alterations. Mol Microbiol **2002**; 46(5): 1295-304.
199. Escribano I, Rodriguez JC, Llorca B, Garcia-Pachon E, Ruiz M, Royo G. Importance of the efflux pump systems in the resistance of Mycobacterium tuberculosis to fluoroquinolones and linezolid. Chemotherapy **2007**; 53(6): 397-401.
200. Velayati AA, Farnia P, Ibrahim TA, et al. Differences in cell wall thickness between resistant and nonresistant strains of Mycobacterium tuberculosis: using transmission electron microscopy. Chemotherapy **2009**; 55(5): 303-7.
201. Parwati I, van Crevel R, van Soolingen D. Possible underlying mechanisms for successful emergence of the Mycobacterium tuberculosis Beijing genotype strains. Lancet Infect Dis **2010**; 10(2): 103-11.
202. Huang TS, Liu YC, Sy CL, Chen YS, Tu HZ, Chen BC. In vitro activities of linezolid against clinical isolates of Mycobacterium tuberculosis complex isolated in Taiwan over 10 years. Antimicrob Agents Chemother **2008**; 52(6): 2226-7.
203. Louie A, Heine HS, Kim K, et al. Use of an in vitro pharmacodynamic model to derive a linezolid regimen that optimizes bacterial kill and prevents emergence of resistance in Bacillus anthracis. Antimicrob Agents Chemother **2008**; 52(7): 2486-96.
204. Rodriguez JC, Cebrian L, Lopez M, Ruiz M, Jimenez I, Royo G. Mutant prevention concentration: comparison of fluoroquinolones and linezolid with Mycobacterium tuberculosis. J Antimicrob Chemother **2004**; 53(3): 441-4.
205. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis **2011**; 204(12): 1951-9.
206. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis **2012**; 55(2): 169-77.
207. Rayner CR, Baddour LM, Birmingham MC, Norden C, Meagher AK, Schentag JJ. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. Infection **2004**; 32(1): 8-14.
208. Andes D, van Ogtrop ML, Peng J, Craig WA. In vivo pharmacodynamics of a new oxazolidinone (linezolid). Antimicrob Agents Chemother **2002**; 46(11): 3484-9.
209. Boak LM, Li J, Rayner CR, Nation RL. Pharmacokinetic/pharmacodynamic factors influencing emergence of resistance to linezolid in an in vitro model. Antimicrob Agents Chemother **2007**; 51(4): 1287-92.
210. Alffenaar JW, van Altena R, Harmelink IM, et al. Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. Clin Pharmacokinet **2010**; 49(8): 559-65.
211. Wallis RS, Jakubiec WM, Kumar V, et al. Pharmacokinetics and whole-blood bactericidal activity against Mycobacterium tuberculosis of single doses of PNU-100480 in healthy volunteers. J Infect Dis **2010**; 202(5): 745-51.
212. Barry P, Deck D, Farkas A. Pharmacokinetic modeling of linezolid dosing regimens for multidrug resistant tuberculosis. IDWeek 2014.
213. Zhang L, Pang Y, Yu X, et al. Linezolid in the treatment of extensively drug-resistant tuberculosis. Infection **2014**; 42(4): 705-11.
214. Liu Y, Bao P, Wang D, et al. Clinical outcomes of linezolid treatment for extensively drug-resistant tuberculosis in Beijing, China: a hospital-based retrospective study. Jpn J Infect Dis **2015**; 68(3): 244-7.
215. Koh WJ, Kang YR, Jeon K, et al. Daily 300 mg dose of linezolid for multidrug-resistant and extensively drug-resistant tuberculosis: updated analysis of 51 patients. J Antimicrob Chemother **2012**; 67(6): 1503-7.
216. Liu H, Wang Y, Liu N, Zhao L. In vitro activities of linezolid against clinical isolates of Mycobacterium tuberculosis from Shenyang, north of China. Indian J Med Microbiol **2015**; 33 Suppl: 164-5.
217. Wallis RS, Wang C, Doherty TM, et al. Biomarkers for tuberculosis disease activity, cure, and relapse. Lancet Infect Dis **2010**; 10(2): 68-9.
218. Wallis RS, Vinhas SA, Johnson JL, et al. Whole blood bactericidal activity during treatment of pulmonary tuberculosis. J Infect Dis **2003**; 187(2): 270-8.
219. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs **2014**; 74(8): 839-54.
220. Alffenaar JW, Kosterink JG, van Altena R, van der Werf TS, Uges DR, Proost JH. Limited sampling strategies for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. Ther Drug Monit **2010**; 32(1): 97-101.

221. Dong HY, Xie J, Chen LH, Wang TT, Zhao YR, Dong YL. Therapeutic drug monitoring and receiver operating characteristic curve prediction may reduce the development of linezolid-associated thrombocytopenia in critically ill patients. Eur J Clin Microbiol Infect Dis **2014**; 33(6): 1029-35.
222. Matsumoto K, Shigemi A, Takeshita A, et al. Analysis of thrombocytopenic effects and population pharmacokinetics of linezolid: a dosage strategy according to the trough concentration target and renal function in adult patients. Int J Antimicrob Agents **2014**; 44(3): 242-7.
223. Bolhuis MS, van Altena R, van Hateren K, et al. Clinical validation of the analysis of linezolid and clarithromycin in oral fluid of patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother **2013**; 57(8): 3676-80.
224. Vu DH, Alffenaar JW, Edelbroek PM, Brouwers JR, Uges DR. Dried blood spots: a new tool for tuberculosis treatment optimization. Curr Pharm Des **2011**; 17(27): 2931-9.
225. Vu DH, Bolhuis MS, Koster RA, et al. Dried blood spot analysis for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother **2012**; 56(11): 5758-63.
226. Cattaneo D, Alffenaar JW, Neely M. Drug monitoring and individual dose optimization of antimicrobial drugs: oxazolidinones. Expert Opin Drug Metab Toxicol **2016**; 12(5): 533-44.
227. Furin JJ, Du Bois J, van Brakel E, et al. Early bactericidal activity of AZD5847 in pulmonary tuberculosis. 46th Union World Conference on Lung Health. Cape Town, **2015**.
228. Wallis RS, Maeurer M, Mwaba P, et al. Tuberculosis-advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis **2016**; 16(4): e34-46.
229. Alffenaar JW, van der Laan T, Simons S, et al. Susceptibility of clinical Mycobacterium tuberculosis isolates to a potentially less toxic derivate of linezolid, PNU-100480. Antimicrob Agents Chemother **2011**; 55(3): 1287-9.
230. Williams KN, Brickner SJ, Stover CK, et al. Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. Am J Respir Crit Care Med **2009**; 180(4): 371-6.
231. Wallis RS, Dawson R, Friedrich SO, et al. Mycobactericidal activity of sutezolid (PNU-100480) in sputum (EBA) and blood (WBA) of patients with pulmonary tuberculosis. PloS one **2014**; 9(4): e94462.
232. Wallis RS, Jakubiec W, Kumar V, et al. Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. Antimicrob Agents Chemother **2011**; 55(2): 567-74.
233. McGee B, Dietze R, Hadad DJ, et al. Population pharmacokinetics of linezolid in adults with pulmonary tuberculosis. Antimicrob Agents Chemother **2009**; 53(9): 3981-4.
234. Mouton JW, Ambrose PG, Canton R, et al. Conserving antibiotics for the future: new ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective. Drug Resist Updat **2011**; 14(2): 107-17.
235. Pasipanodya J, Gumbo T. An oracle: antituberculosis pharmacokinetics-pharmacodynamics, clinical correlation, and clinical trial simulations to predict the future. Antimicrob Agents Chemother **2011**; 55(1): 24-34.
236. Lessem E, Cox H, Daniels C, et al. Access to new medications for the treatment of drug-resistant tuberculosis: patient, provider and community perspectives. Int J Infect Dis **2015**; 32: 56-60.
237. World Health Organization. Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). Geneva, **2018**.
238. Wasserman S, Meintjes G, Maartens G. Linezolid in the treatment of drug-resistant tuberculosis: the challenge of its narrow therapeutic index. Expert Rev Anti Infect Ther **2016**; 14(10): 901-15.
239. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med **2005**; 352(21): 2211-21.
240. World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. Geneva, **2018**.
241. Stalker DJ, Jungbluth GL. Clinical pharmacokinetics of linezolid, a novel oxazolidinone antibacterial. Clin Pharmacokinet **2003**; 42(13): 1129-40.
242. Tsuji Y, Holford NH, Kasai H, et al. Population pharmacokinetics and pharmacodynamics of linezolid-induced thrombocytopenia in hospitalized patients. Br J Clin Pharmacol **2017**;83(8):1758-1772.
243. Sisson TL, Jungbluth GL, Hopkins NK. Age and sex effects on the pharmacokinetics of linezolid. Eur J Clin Pharmacol **2002**; 57(11): 793-7.
244. Deshpande D, Srivastava S, Pasipanodya JG, et al. Linezolid for Infants and Toddlers With Disseminated Tuberculosis: First Steps. Clin Infect Dis **2016**; 63(suppl 3): S80-s7.

245. Olayanju O, Esmail A, Limberis J, Gina P, Dheda K. Linezolid interruption in patients with fluoroquinolone-resistant tuberculosis receiving a bedaquiline-based treatment regimen. Int J Infect Dis **2019**; 85: 74-9.
246. Takahashi Y, Takesue Y, Nakajima K, et al. Risk factors associated with the development of thrombocytopenia in patients who received linezolid therapy. J Infect Chemother **2011**; 17(3): 382-7.
247. Bigelow KM, Deitchman AN, Li SY, et al. Pharmacodynamic Correlates of Linezolid Activity and Toxicity in Murine Models of Tuberculosis. J Infect Dis **2021**; 223(11): 1855-64.
248. Bigelow KM, Tasneen R, Chang YS, Dooley KE, Nuermberger EL. Preserved Efficacy and Reduced Toxicity with Intermittent Linezolid Dosing in Combination with Bedaquiline and Pretomanid in a Murine Tuberculosis Model. Antimicrob Agents Chemother **2020**; 64(10).
249. Wasserman S, Denti P, Brust JCM, et al. Linezolid Pharmacokinetics in South African Patients with Drug-Resistant Tuberculosis and a High Prevalence of HIV Coinfection. Antimicrob Agents Chemother **2019**; 63(3):e02164-18.
250. Kamp J, Bolhuis MS, Tiberi S, et al. Simple strategy to assess linezolid exposure in patients with multi-drug-resistant and extensively-drug-resistant tuberculosis. Int J Antimicrob Agents **2017**; 49(6):688-694.
251. Abdelwahab MT, Wasserman S, Brust JCM, et al. Linezolid population pharmacokinetics in South African adults with drug-resistant tuberculosis. Antimicrob Agents Chemother **2021**; 65(12):e0138121.
252. Cherry CL, Wesselingh SL, Lal L, McArthur JC. Evaluation of a clinical screening tool for HIV-associated sensory neuropathies. Neurology **2005**; 65(11): 1778-81.
253. World Health Organization. Consultation on development of standards for characterization of vision loss and visual functioning. Geneva, **2003**.
254. Lee JK, Lee JY, Kim DK, et al. Substitution of ethambutol with linezolid during the intensive phase of treatment of pulmonary tuberculosis: a prospective, multicentre, randomised, open-label, phase 2 trial. Lancet Infect Dis **2019**; 19(1): 46-55.
255. Hu FB, Goldberg J, Hedeker D, Flay BR, Pentz MA. Comparison of population-averaged and subject-specific approaches for analyzing repeated binary outcomes. Am J Epidemiol **1998**; 147(7): 694-703.
256. Daniels B. CROSSFOLD: Stata module to perform k-fold cross-validation. Statistical Software Components **2012**.
257. McZgee VE, Carleton WT. Piecewise Regression. J Am Stat Assoc **1970**; 65(331): 1109-24.
258. Muggeo VM. Estimating regression models with unknown break-points. Stat Med **2003**; 22(19): 3055-71.
259. Dempsey SP, Sickman A, Slagle WS. Case Report: Linezolid Optic Neuropathy and Proposed Evidenced-based Screening Recommendation. Optom Vis Sci **2018**; 95(5): 468-74.
260. TB Alliance. Pretomanid FDA Sponsor Briefing Document. FDA, **2019**.
261. Robson SC, White NW, Aronson I, Woollgar R, Goodman H, Jacobs P. Acute-phase response and the hypercoagulable state in pulmonary tuberculosis. Br J Haematol **1996**; 93(4): 943-9.
262. Baynes RD, Bothwell TH, Flax H, et al. Reactive thrombocytosis in pulmonary tuberculosis. J Clin Pathol **1987**; 40(6): 676-9.
263. Minchella PA, Donkor S, Owolabi O, Sutherland JS, McDermid JM. Complex Anemia in Tuberculosis: The Need to Consider Causes and Timing When Designing Interventions. Clin Infect Dis **2015**; 60(5): 764-72.
264. Scotton P, Fuser R, Torresan S, et al. Early linezolid-associated lactic acidosis in a patient treated for tuberculous spondylodiscitis. Infection **2008**; 36(4): 387-8.
265. Imperial MZ, Nedelman JR, Conradie F, Savic RM. Proposed linezolid dosing strategies to minimize adverse events for treatment of extensively drug-resistant tuberculosis. Clin Infect Dis **2021**; ciab699. doi: 10.1093/cid/ciab699. Online ahead of print.
266. Little RJ, Rubin DB. Statistical analysis with missing data: John Wiley & Sons, **2019**.
267. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. The Journal of infectious diseases **2013**; 208(9): 1464-73.
268. Chigutsa E, Pasipanodya JG, Visser ME, et al. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. Antimicrob Agents Chemother **2015**; 59(1): 38-45.
269. Zong Z, Jing W, Shi J, et al. Comparison of in vitro activity and MIC distributions between the novel oxazolidinone delpazolid and linezolid against multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis in China. Antimicrob Agents Chemother **2018**; 62(8):e00165-18.

270. Pang Y, Lu J, Huo F, et al. Prevalence and treatment outcome of extensively drug-resistant tuberculosis plus additional drug resistance from the National Clinical Center for Tuberculosis in China: A five-year review. J Infect **2017**; 75(5): 433-40.
271. Zhang S, Chen J, Cui P, et al. Mycobacterium tuberculosis Mutations Associated with Reduced Susceptibility to Linezolid. Antimicrob Agents Chemother **2016**; 60(4): 2542-4.
272. Zimenkov DV, Nosova EY, Kulagina EV, et al. Examination of bedaquiline- and linezolid-resistant Mycobacterium tuberculosis isolates from the Moscow region. J Antimicrob Chemother **2017**; 72(7):1901-1906.
273. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of biomedical informatics **2009**; 42(2): 377-81.
274. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis **2010**; 10(9): 621-9.
275. Ismail NA, Mvusi L, Nanoo A, et al. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. Lancet Infect Dis **2018**; 18(7): 779-87.
276. de Lamballerie X, Zandotti C, Vignoli C, Bollet C, de Micco P. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res Microbiol **1992**; 143(8): 785-90.
277. Klitgaard RN, Ntokou E, Norgaard K, et al. Mutations in the bacterial ribosomal protein l3 and their association with antibiotic resistance. Antimicrob Agents Chemother **2015**; 59(6): 3518-28.
278. Ismail N, Omar SV, Ismail NA, Peters RPH. Collated data of mutation frequencies and associated genetic variants of bedaquiline, clofazimine and linezolid resistance in Mycobacterium tuberculosis. Data Br **2018**; 20: 1975-83.
279. Schmalstieg AM, Srivastava S, Belkaya S, et al. The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob Agents Chemother **2012**; 56(9): 4806-15.
280. Zhao Y, Fox T, Manning K, et al. Improved treatment outcomes with bedaquiline when substituted for second-line injectable agents in multidrug resistant tuberculosis: a retrospective cohort study. Clin Infect Dis **2018**; 68(9):1522-1529.
281. Hanekom M, Streicher EM, Van de Berg D, et al. Population structure of mixed Mycobacterium tuberculosis infection is strain genotype and culture medium dependent. PloS one **2013**; 8(7): e70178.
282. Metcalfe JZ, Streicher E, Theron G, et al. Cryptic Microheteroresistance Explains Mycobacterium tuberculosis Phenotypic Resistance. Am J Respir Crit Care Med **2017**; 196(9): 1191-201.
283. Conradie F, Everitt D, Olugbosi M, Wills G, Fabiane S, Timm J, Spigelman M. High rate of successful outcomes treating highly resistant TB in the ZeNix study of pretomanid, bedaquiline and alternative doses and durations of linezolid. In: IAS, 2021.
284. Baietto L, D'Avolio A, Ariaudo A, et al. Development and validation of a new UPLC-PDA method to quantify linezolid in plasma and in dried plasma spots. J Chromatogr B Biomed Appl **2013**; 936: 42-7.
285. Kim HY, Ruiter E, Jongedijk EM, et al. Saliva-based linezolid monitoring on a mobile UV spectrophotometer. J Antimicrob Chemother **2021**; 76(7): 1786-92.
286. Wasserman S, Davis A, Wilkinson RJ, Meintjes G. Key considerations in the pharmacotherapy of tuberculous meningitis. Expert Opin Pharmacother **2019**;20(15):1791-1795.
287. Viaggi B, Paolo AD, Danesi R, et al. Linezolid in the central nervous system: comparison between cerebrospinal fluid and plasma pharmacokinetics. Scand J Infect Dis **2011**; 43(9): 721-7.
288. Luque S, Grau S, Alvarez-Lerma F, et al. Plasma and cerebrospinal fluid concentrations of linezolid in neurosurgical critically ill patients with proven or suspected central nervous system infections. Int J Antimicrob Agents **2014**; 44(5): 409-15.
289. Shoen C, DeStefano M, Hafkin B, Cynamon M. In Vitro and In Vivo Activities of Contezolid (MRX-I) against Mycobacterium tuberculosis. Antimicrob Agents Chemother **2018**; 62(8):e00493-18.