Efficacy and Safety of Novel and Repurposed Drugs for the Treatment of Drug-Resistant Tuberculosis

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PhD Declaration

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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 thesis, and where co-authorships are involved, my co-authors have agreed that I may include the following publications:

1. Publication 1: Long-term bedaquiline-related treatment outcomes in patients with extensively drug-resistant tuberculosis from South Africa.
2. Publication 2: Linezolid interruption in patients with fluoroquinolone-resistant tuberculosis receiving a bedaquiline-based treatment regimen.
3. Publication 3: A regimen containing bedaquiline and delamanid compared to bedaquiline in patients with drug resistant tuberculosis.

Olatunde Olayanju

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Details of Publications Included in this thesis

Publication	Journal Impact Factor	Citation Counts	Awards
Olayanju O, Limberis J, Esmail A, Suzette Oelofse, Phindile Gina, Elize Pietersen, Mohammed Fadul, Rob Warren and Keertan Dheda. Long-term bedaquiline-related treatment outcomes in patients with extensively drug-resistant tuberculosis from South Africa. Eur Respir J 2018; 51:1800544 [https://doi.org/10.1183/13993003.00544-2018].	12.242	35	South African Medical Research Council (SAMRC) travel grant for presentation at PATS and SATS Congress 2018. MECOR Graduate Award for presentation at American Thoracic Society (ATS) Conference (2018) Faculty of Health Sciences (UCT) Best Publication Award (2018)
Olayanju O, Esmail A, Limberis J, Gina P, and Keertan D. Linezolid interruption in patients with fluoroquinolone-resistant tuberculosis receiving a bedaquiline-based treatment regimen. International Journal of Infectious Diseases 2019 (85), 74-79.	3.538	4	MECOR Graduate Award for presentation at American Thoracic Society (ATS) Conference (2019)
Olayanju O, Esmail A, Limberis J, and Keertan Dheda. A regimen containing bedaquiline and delamanid compared to bedaquiline in patients with drug resistant tuberculosis. Eur Respir J 2019; https://doi.org/10.1183/13993003.01181-2019].	12.242	1	None yet

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Abstract

Background: There is widespread concern about the rise of drug-resistant TB because treatment outcomes of affected patients remain poor and treatment options are limited. After more than a forty-year gap without any breakthrough discovery, several new (bedaquiline and delamanid) and repurposed drugs (linezolid) are increasingly becoming available for use. However, data regarding the efficacy and safety of these drugs in drug-resistant TB patients, with or without HIV infection, from a real-life programmatic setting are lacking. This thesis aims to address that knowledge gap and provide information for management of drug-resistant TB in countries with high disease burden.

Methods: A total of 326 drug resistant TB patients were prospectively followed up between January 2008 and April 2018. The efficacy and safety of two new drugs (bedaquiline and delamanid) and one repurposed drug (linezolid) was determined in these patients in three studies. In the first study, 24 months treatment outcomes and adverse event profiles were compared between extensively drug resistant (XDR) TB patients who received programmatic treatment regimens with the backbone of second line injectables and fluoroquinolones (non-bedaquiline-based) and those who received a bedaquiline- and/ or linezolid-based treatment regimen. The second study determined the frequency of system-specific adverse events associated with linezolid. The third study interrogated the safety and effectiveness of a strengthened treatment regimen containing a combination of delamanid and bedaquiline in patients with poor prognostic features compared to bedaquiline-based regimen.

Results: In the first study, patients who received a bedaquiline-based treatment regimen had a significantly greater favourable outcome rate (66.2% vs 13.2%; $p<0.001$), more than a four-fold reduction in treatment failure rate (5.9% vs 26%; $p<0.001$) and less than a half of mortality rate compared to patients who received a non-bedaquiline-based regimen. The bedaquiline

survival and favourable outcome effect remained significant in HIV-infected patients ($p<0.001$).

The second study showed that linezolid interruption was common in patients receiving a bedaquiline-based treatment regimen, and that system-specific toxicity occurred within predictable time frames. It also showed that anaemia (77.3% *versus* 7.3%; $p<0.001$), peripheral neuropathy (63.6% *versus* 14.6%; $p=0.003$), and optic neuritis (18.2% *versus* 9.8%; $p=0.34$) occurred more frequently in linezolid interrupters than in non-interrupters.

The third study showed that the use of delamanid-bedaquiline combination regimen was safe and efficacious in drug resistant TB patients with poor prognosis when compared with outcomes in the less sick patients who received a bedaquiline-based regimen. It also showed no significant difference in culture conversion rate at 6 months (92.5% versus 81.8%; $p=0.26$) or favourable treatment outcome rate (63.4% versus 67.5%; $p=0.66$) between the two groups. Although patients who received the combination regimen had more frequent occurrence of QTcF prolongation greater than 60 ms from baseline ($p=0.001$) and more episodes of QTcF greater than 450 ms during treatment ($p=0.001$), none of them were symptomatic or had delamanid or bedaquiline withdrawn from their regimen.

Conclusion: These data demonstrated that new and repurposed drugs remarkably improved treatment outcomes in patients with drug-resistant TB. Although linezolid, which is an important component of the bedaquiline-based treatment regimen, is often associated with system-specific adverse events, these occurred at predictable time frames thereby guiding physicians to make informed management decisions. Lastly, drug resistant TB patients with poor prognosis may benefit from a regimen containing delamanid and bedaquiline which seems relatively safe from an adverse event perspective. These data, despite some limitations, make

a case for a widespread and accelerated roll-out of new and repurposed drugs for the treatment of drug resistant TB.

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Abbreviations

AE	Adverse event
AME	Aminoglycoside modifying enzymes
Bdq	Bedaquiline
CD4	Cluster of differentiation 4
CI	Confidence interval
DNA	Deoxyribonucleic acid
DoH	Department of Health, South Africa
DOTS	Directly Observed Therapy Short course
DR-TB	Drug-resistant tuberculosis
DST	Drug susceptibility testing
ECG	Electrocardiogram
FDA	Food and drug administration
FQ	Fluoroquinolone
HAART	Highly active anti-retroviral therapy
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
INH	Isoniazid
IQR	Interquartile range
LAPS	Linezolid-related adverse events predictive score
LJ	Lowenstein-Jensen
LPA	Line probe assay line probe assay
LTFU	Lost to follow-up
Lzd	Linezolid
MCC	Medicines Control Council

MDR	Multi-drug resistant
MGIT	Mycobacteria growth inhibitor tube system
MIC	Minimum inhibitory concentration
MLSB	Macrolides, Lincosamide and Streptogramin B group of antibiotics
MODS	Microscopic Observation Drug Susceptibility
MTB	*Mycobacteria tuberculosis*
NPV	Negative predictive value
PAS	Para-amino salicylic acid
PCR	Polymerase chain reaction
PPV	Positive predictive value
Pre XDR-TB	Pre-extensively drug-resistant tuberculosis
PZA	Pyrazinamide
RIF	Rifampicin
RR-TB	Rifampicin-resistant tuberculosis
SAHPRA	South Africa Health Products Regulatory Authority
SDG	Sustainable development goal
SLI	Second line injectables
SMG	Smear grade
STREAM	Standard Treatment Regimen of Anti-Tuberculosis drugs for MDR-TB
TB	Tuberculosis
TTP	Time to culture positivity
UN	United Nations
WHO	World Health Organisation
WRD	WHO-recommended rapid diagnostic
XDR-TB	Extensively drug-resistant tuberculosis

PART I: Introduction and Literature Review

Chapter 1: Introduction

1.1. Background for the study

Drug-resistant tuberculosis (DR-TB) has continued to undermine the global effort to eradicate tuberculosis (TB) in all regions of the world [1]. It has progressively contributed to increased morbidity and mortality figures associated with tuberculosis, and more patients appear to be acquiring the drug-resistant strains leading to difficulty in achieving favourable treatment outcomes [2, 3]. There has been a gradual evolvement of the drug-resistant strains over the years, creating a spectrum from mono-resistant (rifampicin-resistant) strains through to multidrug-resistant strains (defined broadly as resistance to rifampicin, isoniazid and/or fluoroquinolones) and more recently, totally drug-resistant strain of tuberculosis (strains that are resistant to all conventional forms TB pharmacological treatment) [2, 4-7].

Multidrug resistant TB (MDR-TB) is described as the disease caused by a strain of *M. tuberculosis* with resistance to at least rifampicin and isoniazid. MDR-TB strains can develop further resistance to either fluoroquinolones and/or second-line injectable drugs (referred to as extensively drug resistant tuberculosis; XDR-TB) [8]. Furthermore, totally resistant strains of TB have now been described, however, there are controversies surrounding this category due to the poor reproducibility and sub-optimal sensitivity of some of the methods used to determine drug susceptibility [8, 9].

The World Health Organisation (WHO) surveillance on drug resistance TB, having been in operation for over 20 years, has generated data on resistant TB for 160 countries, highlighting the magnitude of the disease spread [1]. In 2017 alone, an estimated 558,000 incident cases of MDR/RR-TB were reported globally. MDR/RR-TB also constituted an estimated 3.5% of new cases and 18% of previously treated cases of tuberculosis in the same year, worldwide [1]. In terms of mortality, about 230,000 deaths were associated with MDR/RR-TB in 2017, and more

than half of those mortalities originated from China, India and Russia which were the three countries with the highest burden of the disease [1].

Patients with M/XDR-TB who fail conventional standardized treatment regimen become therapeutically destitute, and without having any potential of achieving cure, some of these patients get discharged into the community where they tend to survive long enough to transmit highly resistant forms of TB to their family and care givers in their loop of contact [10-12].

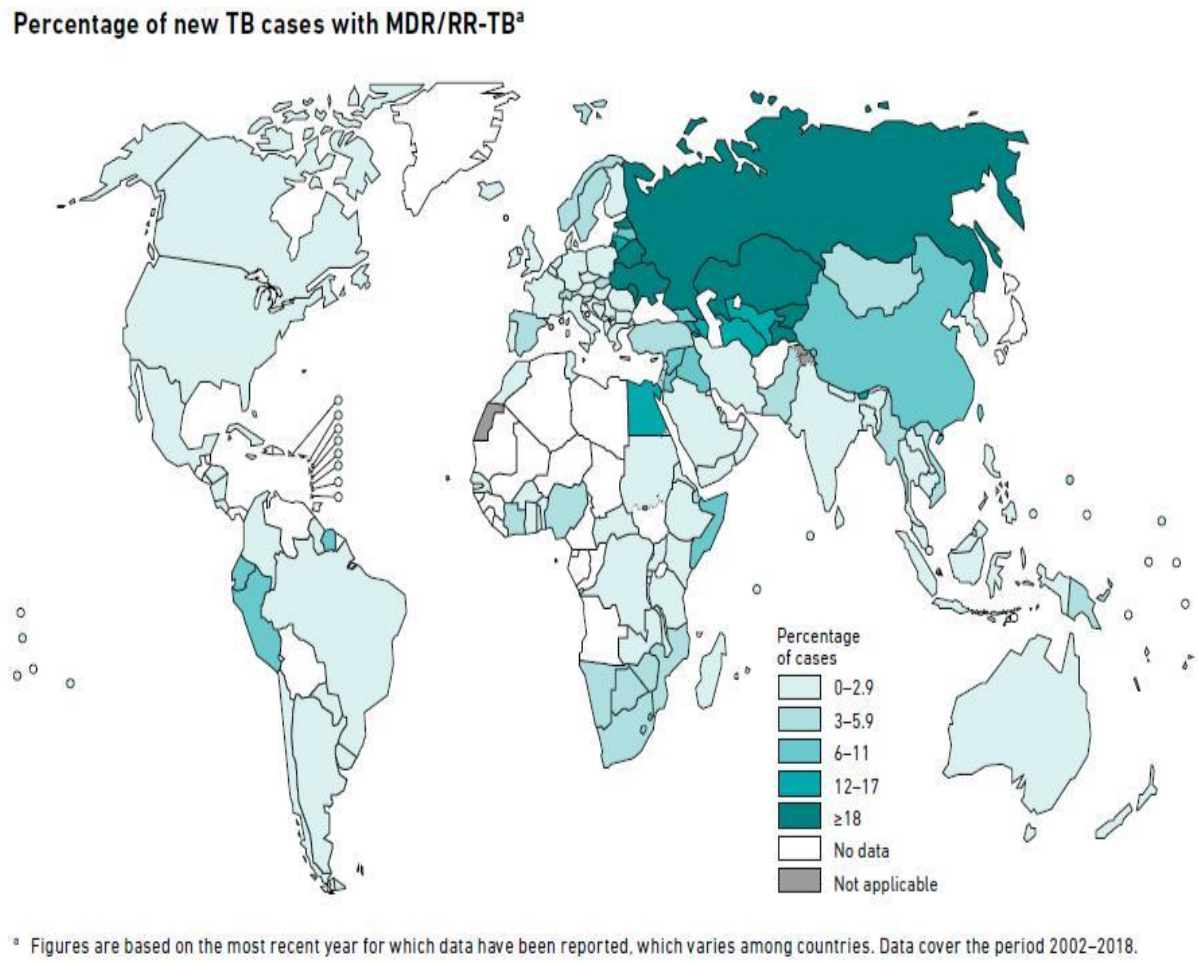

Figure 1.1: Percentage of new cases of MDR/ RR-TB globally (WHO global TB report 2018).

In south Africa, an estimated 3.4% of all new cases and 7.1% of previously treated cases of pulmonary tuberculosis notified in 2017 were MDR/ RR-TB and this was one of the highest rates in the African region [1]. Contrasting these proportions with the 2001 values of 1.8% and

6.7%, respectively, the prevalence of drug-resistant tuberculosis is apparently on the increase in South Africa [13]. However, treatment outcome of these patients remains poor despite the positive political will and huge financial commitment at mitigating the crisis [14-16]. It is on record that the largest outbreak of extensively drug-resistant tuberculosis occurred in Tugella Ferry community in Durban, South Africa in 2005, about 98% of those patients died within a median 16 days of diagnosis [17]. More than a decade later, the success rate for MDR/RR-TB still revolves around 55%, underscoring the poor outcome associated with the disease [18].

Besides the poor outcome rate, the disease burden continues to stretch the budgetary allocations of governments for the diagnosis and treatment of drug-resistant tuberculosis. In 2011, despite constituting only about 2% of all tuberculosis caseload in South Africa, drug-resistant tuberculosis consumed about 45% of national TB budget in the area of diagnosis and treatment [19]. This value has steadily increased over the years, as more than 80% of TB treatment cost was estimated for drug-resistant tuberculosis alone in 2017/2018 by the national government, despite making up less than 10% of total caseload [20].

DR-TB also poses a great threat to health care workers who are at a greater risk of infection. A retrospective study done in South Africa in 2010 showed that health care workers are substantially more likely to be hospitalized for drug-resistant tuberculosis than non-health care workers [21]. A systematic analysis corroborating this high likelihood showed that long period of contact with infected patients, delay in diagnosis and inadequate infection control measures are the major reasons health care worker are very likely to get infected [22]. Not only does this nosocomial infection threaten the life and career of affected health care workers, it is also a threat to the already insufficient number of caregivers available to provide care for infected patients worldwide [23, 24].

Historically, the landscape of treatment guideline for drug resistant tuberculosis has changed repeatedly in the last 20 years, some of the drugs have been shuffled between different treatment groups over the years to ensure a better combination for improved outcome [25-27]. These efforts however yielded very little success because treatment outcomes for drug resistant tuberculosis remain poor. In 2017, only 61% of all RR/MDR-TB patients from the African region, and 55% from South Africa achieved a favourable outcome, despite receiving the recommended treatment [1]. These results informed a continued search for more potent treatment regimen, to which the WHO appropriately responded. The most recent treatment guideline released by the WHO highlighted a major shift and advocates for an all oral longer regimen. It entails a change in the grouping of the drugs based on a new priority ranking to ensure an effective combination [28]. New and repurposed drugs prominently allocated to group A included bedaquiline and linezolid, while delamanid was allocated to group C to complement other priority drugs based on individual patients' susceptibility.

Table 1.1: New WHO recommended treatment guideline for drug resistant TB (WHO, 2018)

Group A Include all three drugs unless they cannot be used	Levofloxacin or moxifloxacin
	Bedaquiline
	Linezolid
Group B Add both drugs unless they cannot be used	Clofazimine
	Cycloserine or terizidone
Group C Add to complete the regimen and when drugs from groups A and B cannot be used	Ethambutol
	Delamanid
	Pyrazinamide
	Imipenem-cilastatin or meropenem
	Amikacin
	Ethionamide or prothionamide
	p-aminosalicylic acid

Several studies have highlighted the efficacy of bedaquiline, linezolid and delamanid, mostly in clinical trials [29-33]. We now know that results from carefully conducted clinical trials are prone to the Hawthorne effect which increases favourable outcome rates and may not be representative of the real-world situations [34]. There are however scarce data regarding the use of these drugs from countries with high disease burden, in a real-life programmatic setting.

In order to address this knowledge gap, three chronological studies were conducted to determine the efficacy and safety profile of these drugs.

1.2. Key research questions

1. What is the treatment outcome and adverse event profile of extensively drug-resistant tuberculosis patients who received a bedaquiline-based treatment regimen compared to those who received a non-bedaquiline-based treatment regimen?

2. What is the frequency of adverse events associated with linezolid use in patients receiving a bedaquiline-based treatment regimen?

3. What is the safety profile and efficacy of a bedaquiline-delamanid combination regimen in drug-resistant tuberculosis patients with poor prognosis compared to the less sick patients who received a bedaquiline-based treatment regimen?

1.3. Aims of the study

Aim 1: To compare the treatment outcome between XDR-TB patients who received a bedaquiline-based treatment regimen and those who received a non-bedaquiline-based treatment regimen.

Sub-aim 1.1: To compare adverse event rate between the two groups during the same period.

Sub-aim 1.2: To describe the treatment outcome and bedaquiline tolerability in HIV infected patients.

Aim 2: To determine the frequency of linezolid-associated adverse events in drug-resistant TB patients receiving a bedaquiline-based treatment regimen.

Aim 3: To compare the treatment outcome and adverse event profile between drug-resistant tuberculosis patients with poor prognosis who received a bedaquiline-delamanid combination regimen and patients who received a bedaquiline-based treatment regimen.

1.4. Overall project description and chronology of studies in this thesis

This project was conducted at the Centre for Lung Infection and Immunity (CLII), University of Cape Town, South Africa, under the supervision of Professor Keertan Dheda. The unit has a multidrug resistant tuberculosis research facility at the Brooklyn Chest Hospital in Cape Town where dedicated staff recruit patients, collect sample and generate data. Brooklyn Chest Hospital (BCH) is the designated drug-resistant tuberculosis in-treatment centre for the Western Cape province of South Africa. The CLII has an excellent working relationship with BCH, with several high impact studies having been conducted at this hospital including early treatment outcome of XDR-TB patients in 2010 [35], long-term outcome of XDR-TB patients in 2014 [36] , and outcome, infectiousness and transmission dynamics of XDR-TB in 2017 [10]. These studies formed the background upon which this project is built, following the availability of new and repurposed drugs for treating drug-resistant tuberculosis.

Three chronological studies were conducted to evaluate the impact of three new and repurposed drugs (bedaquiline, linezolid and delamanid) on the treatment outcomes of drug resistant tuberculosis, and to determine their safety profiles. The first study compared the 24-month treatment outcome of 204 XDR-TB patients from the pre-bedaquiline era to those of 68 XDR-TB patients who received a bedaquiline-based treatment regimen. In view of the toxicity associated with linezolid which is a key component of the current WHO-recommended all-oral regimen, the second study determined the frequency of those toxicities and the impact of treatment interruption on the 63 patients identified. Lastly, the combination of delamanid and bedaquiline in a treatment regimen has not received support because of the potential synergistic cardiac toxicity. However, patients with poor prognosis (e.g. multiple drug resistance, previous treatment failure with a standardized regimen, co-morbidity like HIV co-infection and diabetes) who do not have an adequate treatment option, may be considered for a bedaquiline and delamanid based treatment. Thus, the third study compared the 18-month treatment

outcome and safety profile of 40 drug-resistant tuberculosis patients with poor prognostic features who received the combination (bedaquiline-delamanid) regimen to those of 82 patients receiving a bedaquiline-based regimen.

The coherence of the studies in this thesis is based on the spectrum of patients' categories. The first study compared the outcomes of patients in the bedaquiline era to historical controls (pre-bedaquiline era). The second study examined the potential challenges associated with treatment of DR-TB in the bedaquiline era, while, the third study evaluated the potential treatment option using other new drugs (post bedaquiline era).

In the three studies, words like defaulters and loss-to-follow-up were used repeatedly, these may appear stigmatising and blaming patients. They were so used to distinguish between patients who willingly wanted no further treatment (and researchers were aware of who they were and where they lived) versus those that were lost to follow-up (patient was untraceable). Whilst this work has already been published in peer reviewed journals, and there's no set agreement on the exact terminology, non-stigmatising language will be used in the future to distinguish these two.

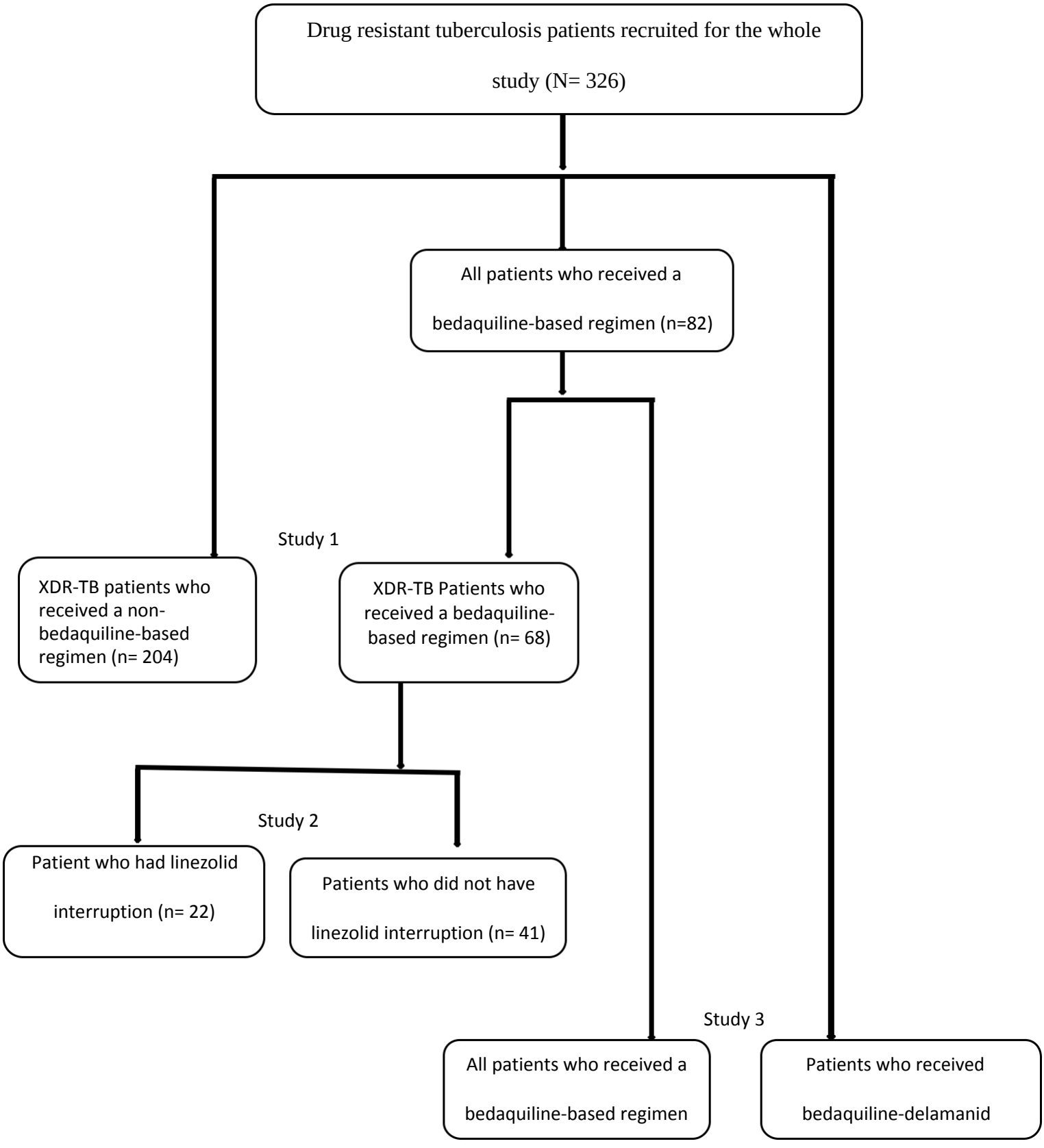

Figure 1.2: Study flow chart showing the coherence of the three studies included in this thesis.

Table 1.2: Relevance of manuscripts to thesis.

Research Question	Study	Key Conclusions	Chapter in thesis
1. What is the treatment outcome and adverse event profile of extensively drug-resistant tuberculosis patients who received a bedaquiline-based treatment regimen compared to those who received a non-bedaquiline-based treatment regimen?	Long-term bedaquiline-related treatment outcomes in patients with extensively drug-resistant tuberculosis from South Africa	XDR-TB patients receiving a backbone of bedaquiline and linezolid had substantially better favourable outcomes compared to those not using these drugs. These data inform the selection of XDR-TB treatment regimens and roll-out of newer drugs in TB-endemic countries.	Chapter 3
2. What is the frequency of adverse events associated with linezolid use in patients receiving a bedaquiline-based treatment regimen?	Linezolid interruption in patients with fluoroquinolone-resistant tuberculosis receiving a bedaquiline-based treatment regimen.	Linezolid-related treatment interruption is common, is strongly associated with HIV co-infection, and system-specific toxicity occurs within predictable time frames. These data inform the clinical management of drug resistant TB patients.	Chapter 4

3. What is the safety profile and efficacy of a bedaquiline-delamanid combination regimen in drug-resistant tuberculosis patients with poor prognosis compared to the less sick patients who received a bedaquiline-based treatment regimen?	A regimen containing bedaquiline and delamanid compared to bedaquiline in patients with drug resistant tuberculosis	Bedaquiline-delamanid combination regimen showed comparable safety and efficacy in the management of drug-resistant TB patients with poor prognosis. These data inform the urgent need to scale-up treatment regimen for drug resistant TB patients	Chapter 5

1.5. Outline of thesis

In chapter 2, I discussed the literature review highlighting the burden of drug-resistant tuberculosis in South Africa and the world. I also reviewed the pathogenesis of drug-resistant tuberculosis, factors associated with the infection, previous treatment guideline, the persistence of poor outcome and the advent of new and repurposed drugs.

In chapter 3, I presented the treatment outcome of XDR-TB patients who received a bedaquiline-based treatment regimen, as compared to the patients who received a non-bedaquiline-based treatment regimen. I also examined the bedaquiline effect on HIV-infected patients regarding the treatment outcome and tolerability with anti-retroviral therapy. In addition, I presented the factors associated with favourable outcome and the predictability of outcome based on culture conversion at specific time during treatment.

In chapter 4, I presented the adverse events associated with linezolid use in patients receiving a bedaquiline-based regimen, the predictability of those adverse events occurring at specific time points during treatments and factors associated with linezolid interruption.

In chapter 5, I presented the efficacy and safety of bedaquiline-delamanid combination therapy in drug-resistant tuberculosis patients with poor prognosis, looking at how the combination could impact their treatment outcome and the comparing their QTc interval changes with patients who received only a bedaquiline-based therapy.

In chapter 6, I presented the unified summary of the three studies, and made a conclusion on how the new and repurposed drugs have impacted the treatment outcome of drug-resistant tuberculosis. I also made recommendations based on the findings towards the roll out of the drugs for drug-resistant tuberculosis patients.

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Chapter 2: Literature review

2.1. Burden of drug-resistant tuberculosis

The emergence of drug-resistant tuberculosis has been the bane of the concerted effort to eradicate TB globally [1]. The resistant strains have been classified into three based on the extent of resistant to the most important drugs used for TB treatment. Rifampicin resistant TB (RR-TB) are the strains with resistance to rifampicin, a first-line TB drug, and this is the first in line of the spectrum. Multidrug resistant TB (MDR-TB) is defined as the TB strains with resistance to rifampicin and isoniazid, the two most powerful anti-TB drugs. They require the use of second line drugs for treatment. Extensively drug resistant TB (XDR-TB) is defined as MDR-TB with further resistance to at least a fluoroquinolone and a second line injectable drug; this definition may be revised anytime soon because second-line injectables are no longer recommended for treating MDR-TB [2].

Almost every country of the world has reported cases of drug resistant TB, although the burden of disease varies from one region to the other with several hotspots identified for each region [3]. The 2018 global TB report showed that the South-Eastern Asia region reported the highest number of MDR/RR-TB with an estimated 99,000 cases. This was closely followed by the European region where an estimated 76,000 cases were reported; the African region had an estimated 39,000 cases. Ironically, only three countries accounted for almost half of the global burden of MDR/RR-TB in 2017 including Russia (10%), China (13%) and India (24%) [3].

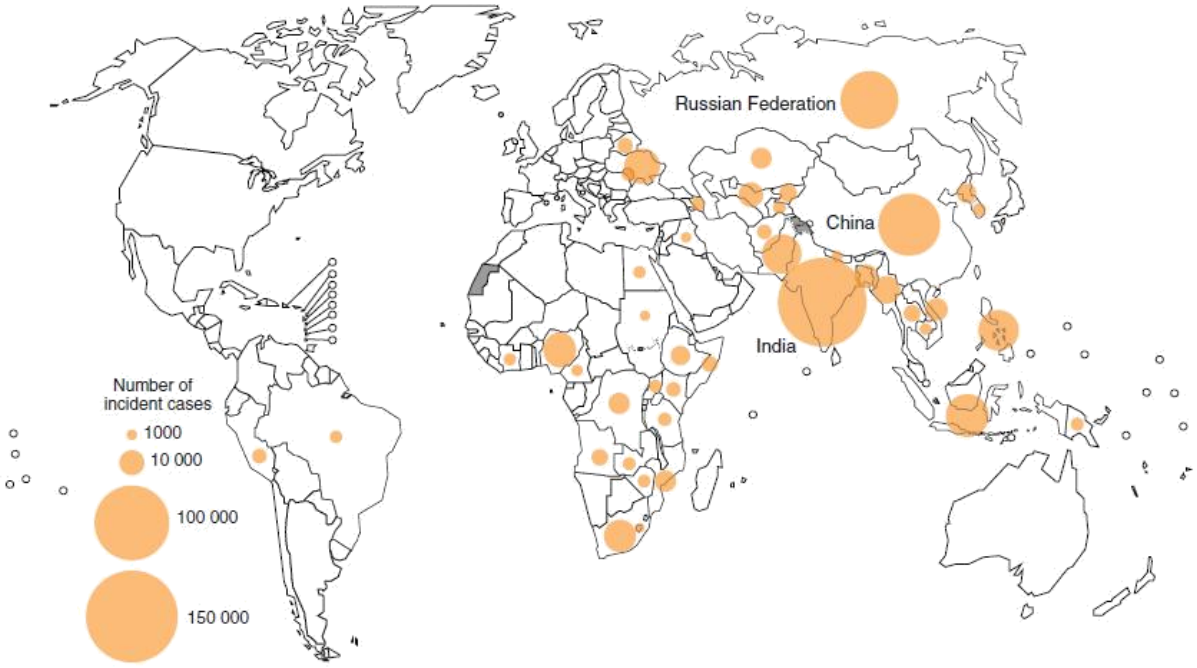

Figure 2.1: Estimated incidence of MDR/RR-TB for countries with at least 1000 incident cases (WHO, Global TB report 2017).

The challenges of drug resistant TB are further worsened by the increasing incidence of XDR-TB globally. The global TB report showed that 7,579 laboratory confirmed cases of XDR-TB occurred in 2015, 8,014 cases occurred in 2016 and 10,800 cases were reported in 2017, globally [3-5]. Despite making up only about 8.5% of all MDR-TB in 2017, XDR-TB cases were reported from 127 member-countries of the World Health Organisation (WHO), an indication of a continuous global spread. These strains are costlier to treat, the drug recommended for their treatment are more toxic, yet treatment outcome of affected patients remains abysmally poor [3].

The African region has continued to report a gradual increase in prevalence of MDR-TB. In the last three years, the number of African countries included in the top 30 countries with MDR-TB disease burden has risen from four to nine [3-5]. This may be an indication that MDR-TB is increasing at a faster rate or decreasing at a slower pace when compared to general TB burden in the countries involved. South Africa prominently features in the list of countries with high

MDR-TB burden every year. Despite huge political goodwill and financial commitment by the government, there has not been an appreciable improvement in the prevalence of MDR-TB over the years, although the TB notifications has been on a downward trend.

The implication of this trend on public health is the potential threat of continuous spread of drug resistant strains of TB to other members of the community where patients reside. There are evidences of primary infection by MDR-TB strains in several literatures and given the high volume of cross-border migrations across the globe, more countries are at risk of this primary infection [6, 7]. More so, the long treatment duration of infected patients in designated centres where they spend substantial period of time with health workers increases the exposure of such care givers and their chances of getting infected [8, 9]. This portends a threat to the already low personnel capacity which will further worsen the chances of patients from getting adequate care.

Despite the increase trend in prevalence of drug-resistant TB, and all the effort directed at improved care, the success rate of affected patients following treatment has remained very low. In 2017, the success rate for MDR/ RR-TB was 55% while it was only 37% for XDR-TB, globally. In the African region, the success rate for MDR/ RR-TB and XDR-TB were 61% and 47%, respectively. The figures were similar for South Africa in the same year with a success rate of 55% and 48% for MDR/ RR-TB and XDR-TB respectively [3].

With prevalence on the upward trend in South Africa, more patients required hospitalisation thereby stretching the available facility. Most of the patients required a long treatment period, and consequently increasing the waiting period for many others. This creates unavoidable delay in treatment initiation and as a result, most of the waiting patients are either lost to follow-up or died while waiting [10]. The South African National Department of Health (DoH) responded to this challenge by decentralising the management of drug resistant TB, empowering the

lower-level facilities to commence the treatment of drug-resistant TB at designated treatment units. This provision was essentially expected to accommodate very sick patients with extensive disease and smear positivity [11, 12]. This however does not appear to reduce the prevalence of the disease substantially despite huge financial and human resources the government committed to the program. There is therefore the need to look for other avenues to improve the outcome of affected patients, especially in the area of drug formulation and creating more effective treatment regimens.

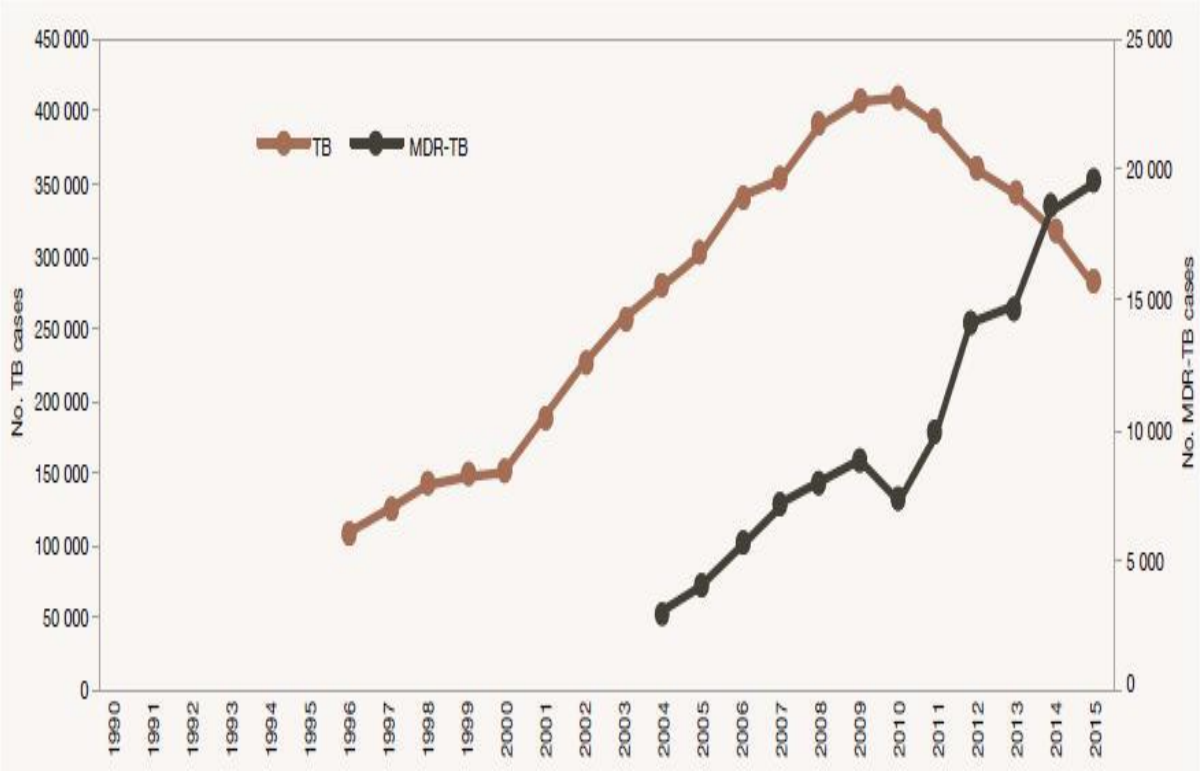

Figure 2.2: Cases of TB and laboratory diagnosed MDR-TB reported between 1996 and 2015 (WHO, 2016).

2.2. Pathogenesis of MDR-TB

The worrisome trend of MDR-TB spread across the globe has generated a lot of discussion regarding the process involved in the transition of drug-sensitive TB to that status which gradually evades treatment until it becomes resistant to multiple available drugs. Several theories have been put forward to explain this change. It is generally believed that poor case management and failure of patients to adhere to treatment schedule are the most likely triggers for developing drug resistance [13-15]. Cases of monotherapy, inappropriate drug combination, and sub-optimal dosing which are all indices of poor management have been associated with development of drug resistance [16, 17]. Mycobacteria regrowth during sub-inhibitory drug concentration and differential bacteriopaucal mechanisms were identified as important stages towards the development of drug resistance. However, in places where deliberate attempts were made to forestall these challenges, cases of drug-resistant TB continued to rise, leading to the search for other possible mechanisms [18, 19].

Establishment of designated treatment centres for drug-resistant TB was targeted at optimising patient's treatment and adherence to therapy. Patients were admitted into these centres and received treatment through the WHO recommended Directly Observed Treatment, Short Course (DOTS) strategy. This also did not appear to stop the development of drug resistance in the patients managed under this programmatic setting [18]. Garcia-Prats and colleagues reported a case of multidrug resistant TB in a patient who received a supervised and recommended combination therapy, there by corroborating the earlier assertion that there is more to developing drug resistance than adherence to treatment guideline [20]. There are currently better understandings beyond treatment adherence, of factors that play important role in developing drug-resistant TB.

2.2.1. Pharmacokinetic variability theory

Due to the differences in the rate at which anti-TB drugs are metabolised in the body, patients are exposed to different concentrations of each components of their treatment regimen over the duration of a single dose of the regimen [21]. Drugs with short half-life are eliminated more quickly from circulation leaving those with prolonged half-life exposed to repeated bacteria replication cycles. This creates an artificial scenario of prolonged monotherapy such that pharmacokinetic variability of components of a treatment regimen predisposes patients to developing resistance to drugs with longer half-life [22]. This assertion has been corroborated by several studies that looked at the association between rate of drug metabolism in individuals and their probability of developing drug resistance. A proteomic study also found that there is a progressive overexpression of certain proteins which are upregulated during the process of resistance development.

Differential drug penetration gradient in lung cavity has also been implicated in the development of drug-resistance [23]. The lung cavity has been shown to be the site where drug resistance is made. The differences in the structural architecture of different positions of lung cavity create a physico-chemical barrier through which antibiotics traverse [23]. Studies have shown that the lowest concentrations of drugs are most commonly found in the centre of body cavities indicating a decline in the drug diffusion along the cavity, which houses the bulk of the mycobacteria [24]. This essentially creates a scenario of sub-optimal dosing of drugs in the region where they are required most, invariably encouraging the development of resistance to those drugs.

2.2.2. Efflux pump theory

The complexity of mycobacteria cell wall creates a potent barrier to the influx of toxic materials into the intracellular space. This is believed to be a mechanism by which several antibiotics are

prevented from penetrating the mycobacteria cell wall, however, hydrophilic compounds are able to diffuse through membrane channels called MspA porins thus creating a leeway for certain drugs to penetrate. However, multiple putative efflux proteins found on the mycobacteria cell wall are actively engaged to maintain the balance between the intracellular and extracellular homeostasis [25]. Some of those efflux pumps are physiologically responsible for getting rid of toxic substances from the intracellular space while some others primarily ensure that anti-TB drugs are extruded, minimising their intracellular concentration and thereby compromising their efficacy [26-28].

Upregulation of these efflux systems during treatment often leads to the loss of effective drug concentration in their intracellular compartment and the continuous exposure to the sub-lethal dose enable them to gradually alter their genetic make-up that favour the development of resistant strains [29, 30]. Srivastava et al demonstrated through a model that the induction of an efflux pump which transport two or more drugs is the first step towards the emergence of resistance. A study suggests that *M.tb* strains are primed to efflux noxious anti-TB drugs, and this was supported by the use of efflux inhibitors like verapamil and chlorpromazine, both of which succeeded in inhibiting isoniazid efflux [31, 32].

Mutations in certain regulatory regions also have been found to cause overexpression of transporters and efflux pumps with capacities to extrude multiple drugs simultaneously. These pumps reduce intracellular antibiotics concentrations to sub-lethal levels thereby promoting the development of resistance [33]. There are studies which have identified efflux pumps for most of the first line anti-TB drugs, making it a very important pathway that needs to be addressed to preserve the activity of these drugs. Some of these efflux pumps affect multiple drugs thereby creating a cross-resistance between several anti-TB drugs. A notable example is the MmpL5 which has been associated with clofazimine resistance and was recently found to be involved in resistance to bedaquiline, a novel anti-TB drug [34].

Table 2.1: List of resistance-encoding mutations genes associated with efflux of anti-tuberculosis drugs.

Drugs	**Efflux gene**
Rifampicin	pstB, Rv1258c, whiB7 [35-37]
Isoniazid	pstB, Rv1258c, mmpL7, iniA, iniB, iniC [35, 37-39]
Ethambutol	pstB, drrA, drrB, drrC, Rv1258c, iniA [37, 40, 41]
Streptomycin	drrA, drrB, drrC[40, 42]
Ofloxacin	Rv1258c [35, 37]
Clofazimine	Rv0678[34, 43]

2.2.3. Genetic mutation theory

Spontaneous mutation has been identified as one of the major pathways by which drug resistance occurs in mycobacteria unlike in other bacteria that possess plasmids or transposons to mediate a horizontal transfer of genetic materials [44, 45]. Specific mutations usually occur at the resistance determining region of target genes in the mycobacteria DNA, consequently altering the structures of specific proteins involved in drug activation. The resulting alteration can manifest as a mismatch at the binding site for drugs rendering them ineffective or disrupt the functions of a regulatory proteins involved in the drug metabolism. The mycobacteria consequently become resistant to the affected drug either at a low or high level depending on the magnitude of expression. A typical example is the katG gene which codes for peroxidase and catalase enzymes activities. These enzymes are involved in activating INH to its bacteriocidal form, therefore, a mutation in the gene leads to a loss of enzymatic activity required for the drug's functionality [46, 47]. Mutations can also occur in promoter and coding

regions of other genes within the mycobacteria DNA resulting in different levels of resistance for one drug as different from another depending on the contribution of such genes to the overall drug activity. Several genetic mutations have been identified to be specifically associated with anti-TB drugs. While the first line and second-line drugs have been well documented, new and repurposed drugs are also joining the list of drugs whose resistance are associated with specific mutations, apparently highlighting the degree of threat mutations contribute to drug resistance. Although it is generally believed that a fitness cost is acquired for each of these mutations, and it is expressed as reduction in mycobacteria growth, virulence and transmissibility, this dogma has been challenged severally. The severity of this fitness cost depends on the gene affected, host factors, environmental factors and further mutations, therefore expression of these mutations may vary from one case to another [48].

Table 2.2: Specific genetic mutation responsible for drug resistance and their mechanisms of action

Drug	**Gene mutation responsible for resistance**	**Mechanism of resistance**
Rifampicin	rpoB	Alteration of drug target [49]
Isoniazid	katG, inhA, ahpC, kasA, ndh	Inhibition of pro-drug activation, alteration of drug target [47, 50]
Pyrazinamide	PncA, rpsA, panD	Inhibition of pro-drug activation [51]
Ethambutol	EmbB, ubiA	Alteration of drug target [52]
Streptomycin	rpsL, rrs, gidB	Alteration of drug target [53]
Ethionamide	ethA, ethR, mabA(fabG)-inhA	Alteration of drug target, Inhibition of pro-drug activation [54]
Fluoroquinolones	gyrA, gyrB	Alteration of drug target [55]
Para-aminosalicylic acid	thyA	Bypassing drug target, Alteration of drug target [56]
Kannamycin, Amicacin, Capreomycin	rrs, tlyA, eis, whiB7	Alteration of drug target [53, 57]
Clofazimine	mmpR	overexpression of efflux pump[34]
Cycloserine	alrA	Alteration of drug target [58]
Bedaquiline	Rv0678, atpE	Alteration of drug target, overexpression of efflux pump [34, 43]
Linezolid	rrl, rplC	Alteration of drug target [59]
Delamanid	ddn, fgd1, fbiA/B/C	Inhibition of pro-drug activation [60]

2.2.4. Modification and inactivation of drugs

Drug modification and inactivation are other common pathways by which mycobacteria elicit drug resistance. These they do by making use of intrinsic intracellular substances which interact with anti-TB drugs and render them ineffective. For example, mycobacteria typically possess *B*- lactamases which destroys the *B*-lactam ring in antibiotics thereby inactivating them [61], these are viable resistant route to penicillins. Other prominent enzymes mycobacteria deploy to inactivate drugs are the aminoglycoside modifying enzymes (AME) which change the structural formation of aminoglycosides by process of acetylation and interfere with their ability to inhibit mycobacteria protein synthesis. TB genome is known to code for other enzymes that inactivate other anti-TB drugs by several other means like phosphorylation, adenylation, glycosylation, etc. [62-64].

The mycobacteria genome is also known to encode for a methyltransferase which has enabled them to avert any serious attack from the macrolide group of antibiotics. Methyltransferase *Erm* are known to show absolute specificity for nucleotide A2058 in 23 S rRNA whose monomethylation confers resistance to certain macrolides, lincosamide and streptogramin B (MLSB) group of antibiotics. A dimethylation at A2058 confers on the other hand confers a high degree of resistance to all MLSB.

Lastly, it has been proven that drug resistant TB can be transmitted primarily from one person to another without a prior infection with the drug susceptible strain. Patients with drug resistant tuberculosis who have previously failed treatment and discharged home for lack of any other treatment option, were implicated in transferring the drug resistant strains to other people in the community. They remained culture positive at the time of discharge and their expectorated cough aerosol sample, collected using CASS within respirable range (<5µm), also turned out culture positive. A whole genome sequencing done was able to identify downstream cases with

identical sequencing profile to the discharged patients, strongly suggesting a community-based transmission [6, 65].

2.3. Diagnosis of drug-resistant tuberculosis

Accurate diagnosis of drug resistant tuberculosis is essential to achieving a successful TB control globally. However, many countries are faced with the challenges of determining the proportion of their TB patients who have resistance to multiple anti-TB drugs. Many affected patients consequently remain undetected and end up not receiving the appropriate treatment. Early detection through drug susceptibility and testing (DST) is essential to determining individual patient's susceptibility status and enabling them to receive effective medications which will improve their treatment outcome. Susceptibility to a particular drug indicates that the patient is likely to do well if given the drug while resistance to the drug tested is an indication that the patients will derive little or no benefit if given such drug in any treatment regimen. Thus, the WHO provided recommendations towards the appropriate testing and guidelines to treating drug resistant tuberculosis [66]. Several laboratory techniques are currently available for diagnosing drug resistant TB, they are broadly referred to as either phenotypic or genotypic.

2.3.1. Phenotypic drug susceptibility testing

This involves the determination of TB growth or its inability to grow on the conventional Lowenstein-Jensen medium containing critical concentration of specific drug that is being tested. The critical concentration for each drug is the lowest concentration of the drug that inhibit the growth of wild strain of *M. tb* that have never been exposed to TB drugs while at the same time not inhibiting strains of M. tb that are considered to be resistant, for example from a patient who is not responding to treatment [67]. Visible growth in this medium provides a qualitative measure of resistance to the drug being tested, while the absence of growth

provides a measure of susceptibility to the drug [68]. A semi-quantitative measure can be introduced to this test by growing the TB inoculum at different ranges of concentration, this can also be used to establish the MIC. This can also be used to determine the degree of resistance such that regulating the dosage can be beneficial to the patient [69].

Quantitative assessment of DST can be done using the indirect proportion method. This method entails the comparison of growth in a drug-containing media relative to that in a drug-free control media. It was validated for use on solid Lowenstein-Jensen (LJ) culture and has remained one of the most popular methods till date [69, 70]. A defined inoculum is introduced into the medium containing the critical concentration of the drug that is being tested, two 10-fold serial dilutions of the inoculum is also introduced into the drug-free control medium. The growth represented by the number of colonies counted on the drug containing medium is expressed as a percentage of that on the drug-free medium. The *M. tb* is said to be resistant to the drug if the colony count on the drug-containing medium is at least 1% of those on the drug-free medium.

Several other methods are available for DST, but they mostly require the use predetermined concentrations of bacillary controls and standardised drug concentrations. The absolute concentration method and the resistance ratio methods are less commonly used but have been reported to show a high level of agreement with the indirect proportion method [71, 72]. Automated liquid-based culture systems are also available for DST. The most commonly used include the Mycobacteria Growth Inhibitor Tube system (MGIT) which has demonstrated excellent efficacy in detecting resistance to TB drugs. It has the added advantage of a more rapid turn-around time of 7-12 days compared to the 28-42 days required for the solid medium platform [69]. This is considered a valuable improvement as it contributes to early diagnosis, potentially reducing morbidity and mortality [73].

More innovative rapid phenotypic DST methods have recently been developed. Microscopic Observation Drug Susceptibility (MODS) assay involves the use of light microscope to visualise characteristic cord formation of *M. tb* in liquid medium [74]. It is able to detect early growth of *M.tb* as strings and tangles in the liquid medium with or without drugs [75]. FASTPlaque-Response, a phage amplication-based test works directly on sputum specimen to detect resistance if growth occurs in samples with drugs [76]. TK medium uses a colorimetric system to detect colour changes following the growth of mycobacteria in culture medium; these characteristics can be used to detect resistant strains [77].

2.3.2. Genotypic drug susceptibility testing

Genotypic drug susceptibility tests are able to detect drug resistance by identifying specific DNA mutations in *M. tb* genome. Even before the patients presents phenotypically, genetic tests are able to predict resistance to specific drug and provide a guide to attending physician to the choice of drug to include in patients' regimen [78]. Due to the rapid nature of the tests, it is recommended that all countries should adopt policies that include diagnostic algorithms in which a WHO-recommended rapid diagnostic (WRD) is the initial diagnostic test for all people with signs and symptoms of TB [79].

The most commonly used WRD test is the Xpert MTB/RIF assay. It is able to simultaneously detect TB and mutations that are predictive of rifampicin resistance. It can be used on a direct sputum specimen, processed sputum sediment and selected extrapulmonary specimen [80]. It is an automated PCR-based test, in which DNA extraction from sample and the analysis are done within the single sample cassette. The test is very simple to use, does not require growth of organism and results are ready within 2 hours. However, it is relatively expensive and there are concerns about the accuracy of the test especially in patients who are paucibacillary or HIV-infected [81]. This has created a challenge in the diagnosis of MDR-TB in patients who are

smear negative and those with extrapulmonary TB. Xpert can also give a false positive result in patients who have phenotypically silent mutation, and this has made some countries like Brazil and South Africa to develop policies regarding confirmatory tests [82, 83]. Measures taken to improve the diagnostic accuracy led to the development of Xpert MTB/RIF Ultra (Xpert Ultra) with a superior sensitivity [84-86]. Xpert Ultra was designed to use an improved assay chemistry and polymerase chain reaction with two multicopy amplification target resulting in a decreased lower limit of detection [87]. However, this improvement came along with loss of specificity in patients with previous TB history [85].

The other genotypic DST recommended by the WHO for detecting multidrug resistant TB is the line probe assay (LPA) [88]. The assay is DNA strip-based test using nucleic acid amplification technique and reverse hybridisation to detect mutations responsible for resistance to rifampicin, isoniazid, fluoroquinolones and second line injectables. It can be used on either clinical specimen or culture isolates. The test is done in three steps including DNA extraction, polymerase chain reaction amplification and reverse hybridisation, each step in different rooms with restricted access and unidirectional workflow [89], thus a high level expertise and laboratory equipment is required. The most commonly used test are the GenoType MTBDRplus for rifampicin and isoniazid, and Geno Type MTBDRsl for second line injectable, fluoroquinolones and ethambutol.

In a systematic review to show the evidence in support of LPAs for rapid detection of resistance, there was a pooled sensitivity of 98.1% (95% C.I: 95.9-99.1) and specificity of 98.7% (C.I :97.3-99.4) for rifampicin across all sample types (sputum and culture isolates). The pooled sensitivity for isoniazid was 84.3% (95% C.I: 76.6- 89.8) and was considered modest, though more variable than the specificity which was 99.9% (95% C.I: 97.5-99.9) [90]. Another study reported an overall concordance of 96% for LPAs when compared with conventional DST for the detection of MDR-TB [91]. When testing cultured isolates for

fluoroquinolone resistance, another systematic review reported a pooled sensitivity of 83.1% (95% C.I: 78.7- 86.7) and a pooled specificity of 97.7% (95% C.I: 94.3- 99.1) compared to DST. In the same review, when smear positive sputum samples were tested, a pooled sensitivity of 85.1% (95% C.I: 71.9- 92.7) and a pooled specificity of 98.2% (C.I: 96.8-99.0) were reported. For second line injectables, when culture isolates were tested, the pooled sensitivities were 87.9% (C.I: 82.1- 92.0) for amikacin, 66.9% (C.I: 44.1- 83.8) for kanamycin and 79.5% (C.I: 58.3- 91.4) for capreomycin. Pooled specificities reported were 99.5% (C.I: 97.5- 99.9) for amikacin, 98.6% (C.I: 96.1- 99.5) for kanamycin and 95.8% (93.4-97.3) for capreomycin. When smear positive sputum samples were tested for resistance to second-line injectables, a pooled sensitivity of 94.4% (C.I: 25.2- 99.9) and specificity of 98.2% (C.I: 88.9-99.7) was reported [92].

The use of LPA has a great potential to enhance early diagnosis of patients with M/XDR-TB. This will provide patents with opportunities to commence treatment early and improved treatment outcomes. WHO has consequently issued a policy statement, endorsing the use of LPA for rapid diagnosis of drug resistant TB and providing guidelines to be considered while using these tests [93]. Countries with high disease burden have been notified of the need to adopt this method, and this is expected to increase in coming years as efforts are being made to make it more available and affordable.

2.4. Treatment guidelines for drug-resistant TB

The treatment guidelines for drug-resistant TB keep changing over the years and this is not unconnected with the unimpressive treatment outcomes observed in patients [94]. In 2016, the WHO divided RR/MDR-TB drugs into four groups (A-D) and recommended that treatment regimen should contain at least five effective drugs with one drug taken from group A, one

from group B, at least two from group C and additional drugs from group D as appropriate [95].

Table 2.3: WHO classification of drugs for drug-resistant TB prior to 2016 update.

Group	**Drugs**
Group 1 (first-line oral drugs)	PZA, Ethambutol, Isoniazid
Group 2 (second-line injectable drugs)	Capreomycin
Group 3 (second-line oral drugs)	Moxifloxacin, Levofloxacin, Ofloxacin
Group 4 (second-line oral drugs)	Terizidone/ cycloserine, Ethionamide, Para-aminosalicylic acid
Group 5 (third-line oral drugs)	Clofazimine, Linezolid, High-dose isoniazid
	Bedaquiline, Amoxycillin-clavulanate
	Thiacetazone, Clarithromycin, Dapsone
	Amoxyl

Table 2.4: WHO classification of second-line anti-tuberculous drugs recommended for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis (2016 update).

Group	**Drugs**
A- Fluoroquinolones	Levofloxacin
	Moxifloxacin
	Gatifloxacin
B- Second line injectables	Amikacin
	Capreomycin
	Kanamycin
	Streptomycin
C- Other second-line agents	Ethionamide or prothionamide
	Cycloserine or terizidone
	Linezolid
	Clofazimine
D- Add-on agents	Pyrazinamide
	Ethambutol
	High dose isoniazid
	Bedaquiline
	Delamanid
	Para-aminosalicylic acid
	Imipenem plus cilastin
	Meropenem
	Amoxycillin plus clavulanate
	Thiacetazone

The choice of drug for constituting the regimen depends on certain factors including age of patient, HIV co-infection, presence of extrapulmonary disease, history of previous exposure to the drug, disease severity and access to reliable DST. A regimen with a backbone of fluroquinolone (group A drug) plus a second-line injectable (group B drug) is recommended

for a minimum of six months, then other drugs are added, and the treatment is expected to last up to 20 months. However, this regimen is associated with a high-level toxicity, pill burden, long duration of daily painful injection which all contribute to poor adherence and unfavourable outcome.

A shorter regimen which is believed to minimize toxicity and enhance adherence was also recommended for selected category of patients. Treatment is expected to last 9-12 months and has been successfully implemented in Bangladesh, Cameroon and Niger with about 90% success rate [96-98]. This regimen comprises kanamycin, prothionamide and high dose isoniazid, to be used for 4-6 months, with moxifloxacin, clofazimine, pyrazinamide and ethambutol given throughout the course of treatment. Contraindications to this regimen include, resistance to any of the drugs except isoniazid, previous exposure to any of the drug for more than one month, intolerance or toxicity to any of the drugs, extrapulmonary disease, pregnancy of drug inaccessibility [99, 100]. This was evaluated in STREAM 1 study and preliminary results could not confirm the non-inferiority of the shorter regimen compared to the longer regimen. However, the WHO is advising national TB programmes to continue using the shorter MDR-TB regimen under the same conditions, while calling for more data to assess its feasibility, effectiveness and safety [101].

2.5. Surgical treatment

Surgical resection is recommended in patients with highly resistant unilateral disease or bilateral apical disease who have failed medical treatment, but still have adequate lung functions [102, 103]. The procedure is believed to reduce the burden of mycobacteria load in the patient and improve the chances of patients' response to further medical treatment. Partial lung resection (lobectomy) was found to be associated with improved treatment success in a group of RR/MDR-TB patients when combine with medical treatment [104]. Other surgical

procedures include segmentectomy, pneumonectomy and pleurectomy, depending on the extent of the disease. Furthermore, there are now options of non-invasive bronchoscopic approaches for selected patients who do not consent or are not fit for surgery [105]. Although there are hardly any data regarding the duration of medical therapy after surgical resection, a minimum of 18-24 months is recommended to ensure optimal therapy.

2.6. New and repurposed drugs

After more than 40 years of stagnation in the development of anti-TB drugs, several new and repurposed drugs are now available for the treatment of drug-resistant tuberculosis. Bedaquiline, linezolid, delamanid, sutezolid, and SQ-109 are some of the drugs being evaluated at the levels of clinical trials and programmatic use in different parts of the world to treat drug-resistant tuberculosis [106]. Some of them are entirely new, and had secured approvals in several countries, while the others are well known antibiotics that have shown impressive performance against drug-resistant strains of TB. By the end of 2017, 68 countries and territories indicated they had started using bedaquiline and 42 reported that they had started using delamanid in their treatment programmes [79].

Bedaquiline is a novel anti-TB drug which belongs to the diarylquinoline group [107, 108]. Its mechanism of action is based on inhibition of ATP synthase, an enzyme central to mycobacteria energy metabolism [109, 110]. It is effective, not only against actively replicating mycobacteria, but also against the non-actively replicating population [109, 111, 112]. Bedaquiline demonstrated a greater level of potency when compared with either rifampicin or isoniazid [113], and it is active against both drug susceptible and drug resistant tuberculosis strains [114]. It was approved by the United States FDA for the treatment of MDR-TB in 2012 based on the report of a phase II clinical trial that showed early culture conversion and improved treatment outcome [115, 116]. However, there were concerns about the safety of its

use due to associated cardiotoxicity and higher mortality rate when compared with the placebo group, leading to stringent conditions attached to its use, as advised by the WHO [117]. More countries have since commenced the inclusion of bedaquiline in MDR-TB treatment regimen and have published preliminary (six months) results [118, 119], this thesis reports a long-term (24 months) treatment outcome and safety profiles associated with bedaquiline use.

Linezolid (Lzd) belongs to the oxazolidinone group of antibiotics, primarily used for treating gram-positive bacterial infections. It inhibits protein synthesis in bacteria by binding the 50s ribosomal subunit and preventing amino acid incorporation [120]. It was approved in 2000 to be administered at 600mg twice daily for a maximum of 28 days. Linezolid was later found to have a high *in vitro* bacteriostatic activity against *Mycobacterium tuberculosis,* including XDR strains [121-123]. Although the adverse events profile following a 28-day therapy for gram-positive bacteria was bearable, the same cannot be said for drug-resistant TB therapy which usually requires a longer treatment duration. Studies have shown that linezolid inclusion in treatment regimen for drug resistant TB is associated with significant cases of myelosuppression, peripheral neuropathy and optic neuropathy [124-126]. Several patients had their dosages reduced, while others had linezolid withdrawn prior to treatment completion because of these adverse events [127].

Despite the apparent toxicities, however, linezolid has demonstrated substantial efficacy following several reports of improved cavity closure, culture conversion and treatment outcome rates in drug resistant-TB patients who received it in their treatment regimen [124, 126, 128], therefore it remains a viable option when designing background regimen in combination with newer drugs like bedaquiline and delamanid [129]. This thesis determined the frequency of adverse events associated with linezolid use in a bedaquiline-based regimen, the time when those adverse events occurred following treatment initiation, and the impact of treatment interruption (linezolid withdrawal or dosage reduction) on treatment outcome.

Delamanid is a nitro-dihydro-imidazooxazole derivative, recently recommended for treating drug-resistant tuberculosis. Its mechanism of action involves the inhibition of the synthesis of mycolic acid, a major component of mycobacterial cell wall. It has shown potent bactericidal activity against both drug susceptible and drug-resistant TB strains, and it is also very effective against both actively replicating and non-actively replicating mycobacteria [130, 131]. In a randomised clinical trial conducted in 17 centres across nine countries, delamanid was associated with a substantial proportion of multidrug-resistant tuberculosis patients achieving culture conversion at two months, when compared to the placebo group [132]. A follow-up report of patients in that trial showed that receiving delamanid for more than 6 months is associated with significant improvement in treatment outcomes and reduction in mortality
[133]. The drug received its first regulatory approval from the European Medicines Agency in 2014 and has been distributed to more than 45 countries afterwards. However, there are concerns about cardiac arrythmias occurring in patients receiving delamanid. This is even more worrisome given that several other drugs used for treating MDR-TB, including moxifloxacin, clofazimine and bedaquiline have QT prolonging effect [134]. The WHO has therefore been cautious about the inclusion of delamanid in treatment regimen containing these drugs, especially bedaquiline, in patients with limited treatment options. This thesis determined the safety and efficacy of treatment regimen containing delamanid and bedaquiline in drug-resistant patients with poor prognostic features, which put them in a position where the combination of these drugs is inevitable.

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PART II: Empirical Research Papers

Chapter Three: Long-term bedaquiline-related treatment outcomes in patients with extensively drug resistant tuberculosis from South Africa

Abstract

Optimal treatment regimens for patients with extensively drug-resistant TB (XDR-TB) remain unclear. Long-term prospective outcome data comparing XDR-TB regimens, with and without bedaquiline (Bdq), from an endemic setting are lacking.

We prospectively followed up 272 South African patients (49.3% HIV-infected; median CD4 169 cells/μl) with newly diagnosed XDR-TB between 2008 and 2017. Outcomes were compared between those who had not received Bdq (pre-2013; n=204) to those who had (post-2013; n=68; 80.9% also received linezolid).

The 24-month favourable outcome rate was substantially better in the Bdq versus the non-Bdq group [66.2% (45/68) versus 13.2% (27/204); p<0.001]. The Bdq group also exhibited reduced 24-month rates of treatment failure (5.9% versus 26.0%; p<0.001) and default (1.5% versus 15.2%; p<0.001). However, linezolid was withdrawn in 32.7% (18/55) of patients in the Bdq group because of adverse events. Admission weight >50kg, an increasing number of anti-TB drugs, and Bdq were independent predictors of survival (the Bdq survival effect remained significant in HIV-infected persons, irrespective of CD4 count).

XDR-TB patients receiving a backbone of Bdq and linezolid had substantially better favourable outcomes compared to those not using these drugs. These data inform the selection of XDR-TB treatment regimens and roll-out of newer drugs in TB-endemic countries.

3.1. Introduction

The persistence of the multi-drug-resistant tuberculosis (MDR-TB) epidemic threatens to destabilise TB control [1, 2]. MDR-TB is defined as a TB strain with resistance to at least isoniazid and rifampicin. In 2016 ~600 000 new cases of MDR- or rifampicin-resistant TB were estimated to have occurred globally. Detection rates have more than doubled in several countries such as China, India and Russia in the last several years, and almost 20% of *Mycobacterium tuberculosis* isolates globally are now resistant to at least one first- or second-line anti-TB drug [3]. Approximately 10% of global MDR-TB strains are thought to be extensively drug-resistant TB (XDR-TB), which is MDR-TB with additional resistance to a fluoroquinolone and a second line injectable drug. These strains may subvert TB control globally because they are associated with high mortality and morbidity, are a major threat to healthcare workers [2, 4], and are unsustainably costly to treat in countries with high TB incidence [5]. In 2016 in South Africa, for example, ~7.1% of patient samples screened were rifampicin resistant or MDR-TB, of which ~8% were XDR-TB [6]. It was estimated that M/XDR-TB will consume over 80% of TB treatment costs in South African in 2017/18 despite MDR-TB making up less than 10% of the total caseload [7] .

Lack of an effective treatment regimen facilitates the person-to-person transmission of XDR-TB even after treatment initiation, and also explains the poor outcomes associated with XDR-TB. The culture conversion rate in patients with XDR-TB between 2002 and 2008 in South Africa was only ~19% by the end of the follow-up period [8] and a prospective follow-up study indicated that only 16% of XDR-TB patients had a favourable outcome [9]. Outcomes were not any better in HIV co-infected XDR-TB patients from the KwaZulu-Natal and Eastern Cape Provinces, with a reported favourable outcome rate of 12.2% in patients receiving ARVs [10].

The advent of new and repurposed bactericidal drugs such as linezolid (Lzd) and bedaquiline (Bdq) have offered new hope for patients with XDR-TB [11-14]. However, Lzd was associated with significant myelo and neurotoxicity mandating the withdrawal of the drug in almost 30% of patients [15, 16]. A phase II(b) study found that Bdq was associated with increased mortality, significant adverse events including QT prolongation and hepatitis, raising concerns about efficacy and outcome [17]. A unified analysis of Bdq in industry-funded clinical trials showed that Bdq was associated with a 24-month failure rate of almost 40% in XDR-TB patients [17]. Moreover, observational datasets from both TB endemic and low burden settings showed encouraging 6-month culture conversion outcomes; however, there are no long-term data [18, 19]. There were also concerns that clofazimine, currently widely used to treat MDR-TB, could potentially induce cross-resistance to Bdq thereby mitigating its potentially favourable impact [20, 21]. Thus, the clear-cut benefit of Bdq in a programmatic setting, remains unclear. Whilst there are limited but encouraging short-term outcome data from endemic settings [22, 23], the lack of long-term (24-month) comparative outcomes means that there remains controversy and equipoise regarding the immediate and widespread roll-out of Bdq to treat XDR-TB versus awaiting results from controlled clinical trials. To address this issue, we compared long-term outcomes using a Bdq- (and often Lzd)-containing XDR-TB regimen, to those not containing Bdq or Lzd, in a high TB incidence setting.

3.2. Methods

3.2.1. Participants

We prospectively followed up 272 patients with laboratory-confirmed XDR-TB who initiated drug therapy, between January 2008 to June 2017 in a programmatic setting (enrolment and follow-up censor dates were April 2016 and June 2017, respectively). 204 patients received a non-Bdq-based anti-TB regimen while 68 received a Bdq-based regimen. All patients were

admitted to Brooklyn Chest Hospital, Cape Town, which is the designated XDR-TB treatment centre in the Western Cape Province of South Africa, and treatment was directly observed by trained health workers. Adverse events were graded and actively reported by medically qualified and experienced attending health care workers using a report form that was attached to every patient's folder (see online supplement table S1 for adverse events grading. Hearing impairment was measured by trained audiologists who conducted testing on all patients as part of the programmatic routine. Demographic and clinical information was obtained by a trained health care worker from patient records and associated healthcare and laboratory systems. The demographic variables we collected were age, gender, and body weight while the clinical variables were HIV status, drugs used in the regimen, adverse events, CD4 count, number of admission days, and ECG results. QTc was corrected using Fridericia's formula and patients with values > 450ms were considered high risk and closely monitored. Upon discharge, treatment was directly observed by trained health workers in local health care facilities closer to patients' homes. Ethical approval was obtained from University of Cape Town human research ethics committee.

3.2.2. Diagnostic Criteria

Of all culture confirmed XDR-TB patients in the Western Cape between 2008 and 2017, only those who initiated treatment were included in the study. Thus, all the included patients had isolates resistant to rifampicin, isoniazid, ofloxacin and amikacin, and fulfilled the criteria for XDR-TB diagnosis. All patients had monthly smear microscopy and culture done during hospitalisation, and sometimes less frequently following hospital discharge.

3.2.3. Treatment regimens

The background 24-month treatment regimen was prescribed by attending physician following the results of individual patient's drug susceptibility testing to isoniazid, rifampicin, ofloxacin and amikacin. XDR-TB patients in the non-Bdq group were treated with a backbone of para-aminosalicylic acid (PAS)/ clofazimine/ capreomycin and second/fourth generation fluoroquinolones (FQs). Capreomycin was used in the hope that high serum levels would have a therapeutic effect and overcome intrinsic resistance; FQ were used since there is differential susceptibility amongst them and most isolates were only tested for resistance to ofloxacin. The other components included pyrazynamide, terizidone, ethionamide etc. The patients who received the Bdq-based treatment regimen often also concurrently received clofazimine, Lzd and levofloxacin (ofloxacin susceptibility testing was performed) as major components of their regimen. HIV-infected patients received ARV, which included lamivudine, nevirapine, efavirenz, tenofovir and abacavir.

3.2.4. Outcomes

Treatment outcomes were assigned according to the adapted 2013 WHO definitions and reporting frameworks for TB, and the proposed core definitions for drug-resistant TB clinical trials recommended by Furin *et al*. (online supplement Table S2) [24, 25]. Patients were said to have achieved culture conversion if they had two consecutive negative sputum culture results, taken at least ~30 days apart (one missing or contaminated culture was allowed between negative cultures, and inability to produce sputum was considered to be a negative result). The treatment outcomes evaluated were cure/treatment completion, deceased, treatment failure, treatment default, and lost to follow-up. Patients who achieved cure/completion were said to have had a favourable outcome while the deceased, defaulted and those who failed treatment were said to have had unfavourable outcomes.

3.2.5. Statistical analysis

The effect of Bdq treatment was determined by comparative analysis of the demographics, clinical records, survival and treatment outcomes. Quantitative and qualitative variables were reported in percentages and median (interquartile range; IQR). Quantitative and qualitative variables were compared using Mann-Whitney U and chi-square or Fisher's exact tests respectively. Kaplan-Meier curves were estimated for the probability of survival from date of diagnosis, and end of follow-up was date of death, date of loss to follow-up, or censor date. Comparisons between strata (e.g., HIV-infected vs HIV-uninfected individuals) were made by the log-rank test. Univariate Cox proportional hazards models were used to estimate the relation between explanatory variables and time-to-event outcomes. Multivariate Cox proportional hazards models included variables that were significantly associated with outcome ($p<0.1$) with clinical relevance and the preselected variable, gender. A p-value of <0.05 was taken as statistically significant. The sensitivity and specificity of sputum cultures to predict outcomes were computed. Statistical analyses were done in R (v3.4.0) using the packages usdm (v1.1.18), corrplot (v0.77), survival (v2.41.3), and survminer (v0.4.0).

3.3. Results

3.3.1. Demographic and clinical characteristics

The non-Bdq group comprised 204 culture confirmed XDR-TB patients admitted between January 2008 and September 2014. Demographic and clinical characteristics are shown in Table 1. Patients were admitted for a median 199 (IQR 77-329) days and received a PAS/ clofazimine/ capreomycin and FQ-based non-Bdq regimen containing a median of 9 (IQR 8-10) drugs (frequencies of drugs are outlined in Table 2). 99/204 (48.5%) patients in this group were HIV-infected with a median CD4 count of 198 (IQR 71-302) cells/µl at admission, and

90/99 (90.9%) had been commenced on anti-retroviral therapy prior to, or within 3 months of diagnosis of XDR-TB.

The Bdq group comprised 68 culture confirmed XDR-TB patients admitted between November 2013 and April 2016. Patients were admitted for a median 158 (102-221) days, they received a Bdq-based regimen which contained a median of 8 (IQR 7-8) drugs (Table 2). Patients received Bdq for a median of 178 (IQR 54-272) days. 35/68 (51.5%) were HIV-infected with a median CD4 count of 146 (IQR 57-271) cells/μl at admission, and they all received anti-retroviral therapy following diagnosis.

3.3.2. Culture conversion

In the non-Bdq group, 67/204 (32.8%) patients achieved culture conversion by the end of 24 months, but only 27/67 (40.3%) of these patients achieved a favourable outcome. The sensitivity of a negative sputum culture to predict survival was 81.0% at 6 months. The specificity of positive sputum culture to predict mortality was also high, reaching 83.6% at 6 months (Table 4).

In the Bdq group, 46/68 (67.6%) patients achieved culture conversion by the end of 24 months and 45/46 (97.8%) of them achieved a favourable outcome. The sensitivity of a negative sputum culture to predict survival was 97.2% at 6 months. The specificity of positive sputum culture to predict mortality at 6 months was 33.3% (Table 4).

3.3.3. Treatment outcomes

A favourable outcome was achieved in only 27/204 (13.2%) patients in the non-Bdq group while the remaining patients had an unfavourable outcome after 24-month follow-up period (Table 5). Only18/99 (18.2%) of HIV-infected patients in this group had a favourable outcome.

A favourable outcome was achieved in 45/68 (66.2%) patients in the Bdq group while the remaining patients had an unfavourable outcome after 24-month follow-up period (Table 5). 24/35 (68.6%) of HIV-infected patients in this group had favourable outcome.

Patients who received Bdq had a higher probability of survival ($p<0.001$; Figure 1A) in time to event analysis. Bdq had a similar effect in HIV-infected patients ($p<0.001$; Figure 1B). Patients in the Bdq group who received anti-retroviral therapy ($p<0.001$) had a significantly higher probability of survival than their counterparts in the non-Bdq group (Figure 1C). Bdq also provided the survival advantage to HIV-infected patients regardless of their CD4 count at admission (Figures 1D and 1E).

3.3.4. Adverse events

486 adverse events were reported by 143/204 (70.1%) patients in the non-Bdq group. Frequencies of adverse events are reported in Table 3. 78/204 (38.2%) patients had at least one drug withdrawn due to adverse events (grade ≥ 3) during treatment. Only 10/78 (12.8%) patients from whom drugs were withdrawn achieved a favourable outcome.

226 adverse events were reported by 65/68 (95.6%) patients in the Bdq group. More patients in this group, 40/68 (58.8%), had at least one drug withdrawn ($p=0.005$), and 23/40 (57.5%) of them achieved a favourable outcome. None of the patients had Bdq withdrawn from the treatment regimen, although 7/68 (10.3%) had a prolonged QT interval within 450-470ms. 5 (71.4%) of these 7 patients achieved a favourable outcome, 1 (14.3%) was lost to follow-up and 1 (14.3%) died. The deceased patient achieved culture conversion after 41 days of Bdq treatment but reverted 61 days later and never achieved another conversion till death; this patient has been on Bdq for 170 days.

3.3.5. Multivariate analysis

Multivariate analysis of patients in both groups suggested that, receiving Bdq (p=0.05; HR=0.24) and number of anti-TB drugs received (p=0.01; HR=0.83) were independent predictors of survival. It also suggested that patients who were HIV-infected (p=0.02; HR=1.51) and those who weighted less than 50kg at admission (p<0.001; HR=1.96), were more likely to die (Table 6I). In HIV-infected patients, receiving Bdq (p=0.01; HR=0.01) and any aminoglycoside (p=0.02, HR=0.06) were independent predictors of survival, and those weighting ≤50kg at admission (p=0.004; HR=2.06) were more likely to die (Table 6II).

3.4. Discussion

To our knowledge this is the first prospective comparative study reporting long-term (24-month) treatment-related outcomes in patients with XDR-TB, treated with and without Bdq, in a TB-endemic setting. These data represent pragmatic and "real world" outcomes as they are derived from a programmatic setting. The key findings of the study were that: (i) favourable outcomes using Bdq (and Lzd) were more than 5-fold better compared to regimens not containing Bdq; (ii) mortality in the Bdq group was more than halved; (iii) treatment failure rates were reduced by more than 4-fold and there was a more than 10-fold reduction in default rates; (iv) Bdq remained an independent predictor of survival (despite the use of Lzd), and other independent outcome predictors included admission weight of more than 50kg (probably reflecting the immune and nutritional status of the patient) and an increasing number of anti-TB drugs used; (v) the Bdq survival and favourable outcome effect remained significant in HIV-infected persons and even at low CD4 counts; (vi) a 6-month negative culture was ~95% predictive of patient survival in the Bdq group, and 81% predictive of a favourable outcome (by contrast, a positive culture at 6 months was highly predictive of death or unfavourable outcome); and (vi) Bdq-related prolonged QT interval occurred in about 10% of the cohort but none had Bdq withdrawn and most still achieved a favourable outcome. By contrast, 33% of patients experienced Lzd withdrawal due to adverse events.

The dominant finding was that Bdq is an independent predictor of survival and favourable outcome, and the backbone of Bdq and Lzd was associated with remarkably better treatment outcomes compared to regimens not containing these drugs. There was also a higher frequency of death in the Bdq group within the first 2 months of treatment initiation (likely due to a survival bias related a higher rate of pre-diagnostic death in the non-Bdq group), however, exclusion of deaths in this early period did not change the study conclusions (see data supplement; Table S10). Concerns regarding QT prolongation and the potential toxicity of Bdq

(reassuringly low in this study) must be compared against the dramatic and exceptional survival improvement in a disease where mortality is ~70% when using a SLI and FQ-based regimen [3], and this raises the question of whether Bdq and Lzd should now be included in all regimens for the treatment of XDR-TB in programmatic settings? Our outcome data are compelling because they allow direct comparison between individuals from the same region who had long-term survival outcomes before and after the introduction of Bdq within the context of a prospective study. By contrast, studies on patients with XDR-TB have, hitherto, reported short-term outcomes only, or those from non-endemic settings. A retrospective study from South Africa [22], an Indian study [23], and a study from KwaZulu-Natal in South Africa [19] reported 6-month culture conversion rates of 76% (n=63), 65% (n=20) and 68% (n=123), respectively, in Bdq-treated patients with XDR-TB. Importantly, the Bdq effect dominated and remained significant, even in HIV-infected individuals and those with low CD4 counts. Nevertheless, our results were inferior to the 24-month 80% favourable outcome rate reported from France in 45 patients where 53% of the cohort had XDR-TB [26]. In our study, more than a third of patients still had unfavourable outcomes and mortality was almost 15% despite Bdq treatment. Firstly, this highlights the poor outcomes associated with XDR-TB (despite Bdq), which is worse than that seen in several common cancers. Secondly, treatment failure still remains a problem. We have previously highlighted the problem of programmatically incurable TB and the substantial longevity of these patients following discharge into the community (given the lack of facilities and bed space, this is the only option available in many TB-endemic countries including India, China, and Russia) [27]. Indeed, in South Africa we are now facing the problem of patients who have failed Bdq and Lzd-based regimens. Only a minority of these patients have access to, or qualify for, surgical lung resection, and it is difficult, if not impossible to construct a salvage regimen for such patients. This highlights the need to protect existing drugs, practice strict antibiotic stewardship, and underscores the need to develop

alternative treatment dosing and delivery strategies that minimise amplification of resistance within TB cavities [28]. Introduction of new and active drugs like carbapenem and delamanid may also be considered to construct effective treatment regimens and protect new drugs, thus limiting the amplification of resistance.

When using a Bdq and Lzd-based regimen for XDR-TB we found that culture negativity at 6 months had an almost 95% predictive value for survival, and an 81% predictive value for a favourable outcome. By contrast, culture positivity at the same time-point was associated with a 100% unfavourable outcome and 50% mortality rate. We believe that this could serve as an important biomarker when evaluating new Bdq-based regimens (if confirmed in prospective studies), or as an early signal to switch to a salvage regimen. These data mirror the findings of Gunther *et al* in MDR-TB where culture negativity at 6-months had a high predictive value for a favourable outcome in MDR-TB using a capreomycin and ofloxacin-based regimen [29].

Several studies have highlighted high toxicity profiles of regimens used to treat drug-resistant TB [30], and concern has been raised about the potential toxicity of Bdq [31]. Ten percent of individuals in our study had a prolonged QT interval but none had to stop the drug. In a systematic review involving 1266 patients, 3.5% discontinued Bdq due to adverse events, and only 0.6% discontinued Bdq because of prolonged QTc interval [32]. There is accumulating experience that Bdq is safe, though published studies have not been powered to detect a small potential mortality increase [14, 33]. Other substantial toxicities were likely related to Lzd. The rate of peripheral neuropathy was almost 4-fold higher than in the non-Bdq group and anaemia was almost 20-fold higher. Indeed, Lzd needed to be stopped in 33% of patients in the Bdq group; nevertheless, patients in this group still had better outcomes notwithstanding the higher rate of drug withdrawal. It is believed that regimens tailored to individual's metabolism will not only reduce Lzd-related toxicity, but also enhance its role in managing XDR-TB [34].The significantly higher portion of patients with hearing impairment in the Bdq arm reflects the

high proportion of patients that were previously treated with aminoglycosides and was not directly related to the drugs used in this regimen.

There are a number of limitations of this study including inclusion bias (patients with severe disease may have died prior to laboratory diagnosis or before treatment initiation). However, our set up was able to capture all patients with a laboratory diagnosis and this bias would have impacted both arms. We did not expressly correct for radiological disease extent at diagnosis (x-rays were non-digitalised and followed patients to their local clinics), however, there were no significant intergroup differences in terms of demographic factors, weight, HIV status (and CD4 count), and microbiological disease severity (smear and time-to-positivity), which are broadly all proxies of disease extent/ severity. Our study was conducted in the Western Cape Province of South Africa, which arguably has better health care infrastructure and lower HIV co-infection rates. Thus, outcomes might be different in settings where the healthcare infrastructure was less developed and where HIV co-infection rates are higher. Almost all the patients in this study were admitted to the designated XDR-TB hospital. It is possible that results may be different in settings where there are no facilities for inpatient treatment reflecting nosocomial transmission and/or a poorer level of care. However, data from MDR-TB decentralisation programmes in South Africa suggest that outcomes are similar to an inpatient setting [35]. Default and loss to follow-up may have impacted the robustness of our data as this was almost 27% in the non-Bdq group. This is likely due to several factors including using an ineffective regimen, and a longer total treatment duration due to the higher rates of previous TB, however, excluding defaulters from the analysis did not change the study conclusions. By contrast, we think that the Bdq outcomes were less likely to have been impacted to a significant extent as default/loss to follow-up rates were lower. Finally, postmortem studies were not performed so that the cause of death could be substantiated. However, this is not practical in a resource-constrained setting, and postmortems studies cannot confirm or refute that the cause of death is drug-related arrhythmia.

In summary, these prospective long-term outcome data from a TB-endemic setting indicate that a Bdq and Lzd-based regimen result in substantial and remarkable improvement in outcomes in patients with XDR-TB. These data inform clinical practice in endemic settings and make a strong case for the immediate and accelerated roll-out of these drugs for the treatment of XDR-TB in endemic settings.

Table 3.1: Comparison of demographic data, clinical characteristics, and treatment outcomes between the bedaquiline and non-bedaquiline groups. Data is n (%) unless otherwise stated.

VARIABLES	Bdq (n = 68)	Non-Bdq (n=204)	p-values
Median age (years)	34.5 (IQR 26-55)	33.5 (IQR 18-73)	0.42
Gender (male)	41 (60.3)	120 (58.8)	0.89
Median body weight at admission (kg)	51.8 (IQR 33.3-78.1)	51.9 (IQR 21.0-89.9)	0.76
Proportion >50kg	39 (57.4)	115 (56.4)	0.89
Previous TB treatment	33 (48.5)	171 (83.8)	<0.001
HIV-infected	35 (51.5)	99 (48.5)	0.81
HIV-infected on ARV	35 (100)	90 (90.9)	0.11
Median CD4 count at admission (µl/ml)	146 (IQR 57-271)	198 (IQR 71-302)	0.51
#Median number of anti-TB drugs received	8 (IQR 7-8)	9 (IQR 8-10)	<0.001
Patients in whom at least one drug was withdrawn due to adverse events	40 (58.8)	78 (38.2)	0.005
Median number of days of admission	158 (IQR 102-221)	199 (IQR 77-329)	0.05
Outcomes			
Favourable (cured/completed treatment)	45 (66.2)	27 (13.2)	<0.001
Unfavourable outcome	23 (33.8)	175 (85.8)	
Deceased	10 (14.7)	69 (33.8)	0.004
Failed	4 (5.9)	53 (26)	<0.001
LTFU	8 (11.8)	22 (10.8)	1
Defaulted	1 (1.5)	31 (15.2)	<0.001
On treatment	0 (0)	2 (1)	–
*Patients with favourable outcome despite drug withdrawal due to adverse events	23 (57.5)	10 (12.8)	<0.001
HIV-infected persons with a favourable outcome	24 (68.6)	18 (18.2)	<0.001

*This was to identify the proportion of patients who had a favourable outcome (regardless of adverse events that necessitated the withdrawal of at least one drug in the treatment regimen); LTFU = Lost to follow-up, #Bdq was included in the total number of anti-TB drugs used in the Bdq group.

Table 3.2: List of drugs used in the bedaquiline and the non-bedaquiline treatment regimens, the proportion of patients who used them, and the frequency of drug withdrawal due to adverse events. Data is n (%) unless otherwise stated.

	Bdq (n=68)		**Non-Bdq (n=204)**		
Drugs	**Patients who received drug**	**Patients in whom drug was withdrawn due to adverse events (grade≥3)**	**Patients who received drug**	**Patients in whom drug was withdrawn due to adverse events (grade≥3)**	**p-values (comparing proportions of patients who received drug)**
Capreomycin	7 (10.3)	6 (85.7)	196 (95.6)	43 (21.9) **	<0.001
Kanamycin	1 (1.5)	1 (100)	110 (53.9)	12 (10.9)	<0.001
Amikacin	0	0	2 (1.0)	0	N/A
#Any aminoglycoside	8 (11.8)	0	202 (99.0)	47	<0.001
Para-amino salicylic acid	64 (94.1)	10 (15.6)	194 (95.1)	13 (6.7)	0.75
Pyrazinamide	66 (97.1)	3 (4.5)	201 (98.5)	10 (5.0)	0.60
Terizidone	61 (89.7)	8 (13.1)	201 (98.5)	10 (5.0)	0.003
Moxifloxacin	13 (19.1)	1 (7.7)	101 (49.5)	3 (3.0)	<0.001
Ofloxacin	0	0	127 (62.3)	3 (2.4)	N/A
Levofloxacin	67 (98.5)	0	0	0	N/A
Ciprofloxacin	0	0	1 (0.5)	0	N/A
##3rd or 4th generation fluoroquinolone	68 (98.5)	0	101 (49.5)	0	<0.001
Clofazimine	67 (98.5)	1 (1.5)	65 (31.9)	2 (3.1)	<0.001
Linezolid	55 (80.9)	18 (32.7)	0	0	N/A
Ethambutol	26 (38.2)	5 (19.2)	189 (92.7)	15 (7.9)	<0.001
Ethionamide	15 (22.1)	6 (40)	198 (97.1)	12 (6.1)	<0.001
High dose isoniazid	22 (32.4)	3 (13.6)	133 (65.2)	13 (9.8)	<0.001
Dapsone	0	0	34 (16.7)	0	N/A
Co-amoxiclavulanate	2 (2.9)	0	79 (38.7)	0	<0.001
Clarithromycin	0	0	43 (21.1)	0	N/A
Amoxycillin	0	0	13 (6.4)	0	N/A
Azithromycin	0	0	1 (0.5)	0	N/A
Meropenem	1 (1.5)	0	0 (0.0)	0	N/A
Bedaquiline	68 (100)	0	0 (0.0)	0	N/A

#combination of amikacin, kanamycin and capreomycin; kanamycin was replaced by capreomycin in the course of the treatment ##treatment with either moxifloxacin or levofloxacin; **significant difference between number of patients from whom drugs were withdrawn.

Table 3.3: List of all adverse events reported in the bedaquiline and the non-bedaquiline group. Data is n (%) unless otherwise stated.

Adverse Event	**Bdq group (N=68)**	**Non-Bdq group (N=204)**	**p-value**
Peripheral neuropathy	15 (22.1)	13 (6.4)	<0.001
Dizziness/disorientation	11 (16.2)	35 (17.2)	0.85
Depression	2 (2.9)	27 (13.2)	0.02
Headache	2 (2.9)	12 (5.9)	0.53
Psychosis	3 (4.4)	17 (8.3)	0.42
Blurred vision	5 (7.4)	5 (2.5)	0.14
Hearing impairment	29 (42.7)	31 (15.2)	<0.001
Tinnitus	1 (1.5)	4 (2.0)	1
Abdominal pain	15 (22.1)	34 (16.7)	0.41
Vomiting	16 (23.5)	58 (28.4)	0.71
Nausea	16 (23.5)	59 (28.9)	0.65
Diarrhoea	6 (8.8)	21 (10.3)	0.91
Acute liver failure	1 (1.5)	6 (2.9)	0.68
Dyspepsia	3 (4.4)	5 (2.5)	0.42
Skin reaction	20 (29.4)	40 (19.6)	0.13
Arthralgia	13 (19.1)	15 (7.4)	0.011
Body pains	19 (27.9)	32 (15.7)	0.04
Anaemia	14 (20.6)	2 (1.0)	<0.001
Deranged renal function	14 (20.6)	41 (20.1)	0.93
Pruritus	3 (4.4)	12 (5.9)	0.77
Hypothyroidism	6 (8.8)	10 (4.9)	0.37
Haematogical disorders	2 (2.9)	2 (1.0)	0.26
Oedema	1 (1.4)	1 (0.5)	0.44
Anxiety	1 (1.5)	N/A	N/A
Sore throat	1 (1.5)	N/A	N/A
Insomnia	0 (0)	4 (2.0)	N/A
Prolonged QT interval	7 (10.3)	N/A	N/A

N/A= not applicable

Table 3.4: Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of culture negativity at specific time points to predict A) survival and B) favourable treatment outcome in each group. See supplementary Table S9 for a combined analysis.

	Sensitivity (%)		**Specificity (%)**		**PPV (%)**		**NPV (%)**		**Number of patients considered**	
Months	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**
A) Survival as dependant variable										
2	82.5	50.0	0	79.0	86.8	54.1	0	76.2	45	121
3	91.5	66.7	33.3	79.5	95.6	66.7	20.0	79.5	50	134
6	97.2	81.0	33.3	83.6	94.6	75.6	50.0	87.5	39	109
12	90.3	75.0	–	84.2	–	75.0	–	84.2	31	62
18	96.2	78.3	–	71.4	–	81.8	–	66.7	26	37
B) Favourable treatment outcome as dependant variable										
2	88.2	60	27.3	75.2	78.9	32.4	42.9	90.5	45	121
3	94.6	72.7	23.1	68.8	77.8	31.4	60.0	92.8	50	134
6	100	94.7	22.2	70.0	81.1	40.0	100	98.4	39	109
12	96.2	100	40.0	74.5	89.3	45.8	66.7	100	31	62
18	100	93.3	50.0	63.6	96	63.6	100	93.3	26	37

Sensitivity = probability that a negative sputum culture will result in patient survival (or in the case of section B, a favourable treatment outcome);

Specificity = probability that a positive sputum culture will result in patient mortality (or in the case of section B, a favourable treatment outcome);

PPV = probability that a patient with a negative sputum culture survived (or in the case of section B, a favourable treatment outcome);

NPV = probability a patient with a positive sputum culture died (or in the case of section B, a favourable treatment outcome).

Table 3.5: Treatment outcomes at specific time-points as measured from treatment initiation. Outcomes were assigned as described in Table S2 (online supplement) for the Bdq (n=68) and non-Bdq (n=204) groups. Data is number of patients (%).

	12 months		**18 months**		**24 months**	
Treatment outcome	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**	**Bdq**	**Non-Bdq**
Favourable	N/A	N/A	N/A	N/A	45 (66.2)	27 (13.2) #
Unfavourable	21 (30.9)	160 (78.4)	23 (33.8)	173 (84.8)	23 (33.8)	175 (85.8) #
Deceased	8 (11.8)	55 (27) *	9 (13.2)	60 (29.4) *	10 (14.7)	69 (33.8) *
Default	2 (2.9)	21 (10.3)	2 (2.9)	26 (12.7) *	1 (1.5)	31 (15.2) #
Treatment failed	5 (7.4)	70 (34.3) #	4 (5.9)	69 (33.8) #	4 (5.9)	53 (26.0) #
LTFU	6 (8.8)	14 (6.9)	8 (11.8)	18 (8.8)	8 (11.8)	22 (10.8)
On treatment	47 (69.1)	44 (21.6)	45 (66.2)	31 (15.2)	0 (0)	2 (1.0)

N/A= Not applicable, p-values were less than *0.05 or #0.005 when comparing time specific treatment outcomes between patients in the Bedaquiline and non-bedaquiline groups. LTFU= Lost to follow-up

Table 3.6: Multivariate Cox proportional hazard model for risk of death in both groups; A) all the XDR-TB patients (n=271), B) HIV-infected patients (n=132). Univariate analyses are shown in supplementary Tables S3 and S4 for the whole cohort and the HIV-infected subgroups, respectively.

Variables	**Hazard ratio (95% C.I.)**	**p-value**
I) All the XDR-TB patients ([A]n=271)		
Weight <50kg at admission	1.96 (1.38,2.78)	<0.001
Gender (male)	1.08 (0.76,1.52)	0.67
[A]HIV-infected	1.51 (1.06,2.15)	0.02
Previous TB treatment	1.08 (0.69,1.68)	0.73
Number of anti-TB drugs received	0.83 (0.72,0.96)	0.01
[B]Bedaquiline	0.24 (0.06,0.98)	0.05
[B]Linezolid	0.43 (0.11,1.61)	0.21
Clofazamine	0.80 (0.47,1.37)	0.42
[C]Third and fourth generation fluoroquinolones	1.10 (0.68,1.76)	0.70
[D]Any aminoglycoside	0.95 (0.24,3.69)	0.94
II) HIV-infected patients ([E]n=132)		
Weight <50kg at admission	2.06 (1.26,3.36)	0.004
Gender (male)	0.73 (0.43,1.23)	0.24
Number of anti-TB drugs received	0.87 (0.67,1.11)	0.26
Any aminoglycoside	0.06 (0.01,0.67)	0.02
On ARV treatment	1.13 (0.44,2.91)	0.80
CD4 count <200 cell/µl X	1.4 (0.85,2.32)	0.19
Bedaquiline	0.01 (0,0.33)	0.01
Linezolid	0.87 (0.1,7.82)	0.90
Clofazamine	0.62 (0.3,1.31)	0.21
Previous TB treatment	1.29 (0.65,2.54)	0.47

A) One patient refused testing; B) 55 of the 68 (80.9%) patients who received bedaquiline also received linezolid. We performed sub-analyses to investigate the effect of linezolid treatment, and to investigate collinear variables (supplementary Table S5). C) 3rd and 4th generation fluoroquinolones = moxifloxacin and levofloxacin; D) Any aminoglycoside = amikacin, capreomycin and kanamycin; E) 2 patients did not have CD4 count done at admission (n=132). X - 31 of the 35 (88.6%) patients who received bedaquiline also received linezolid.

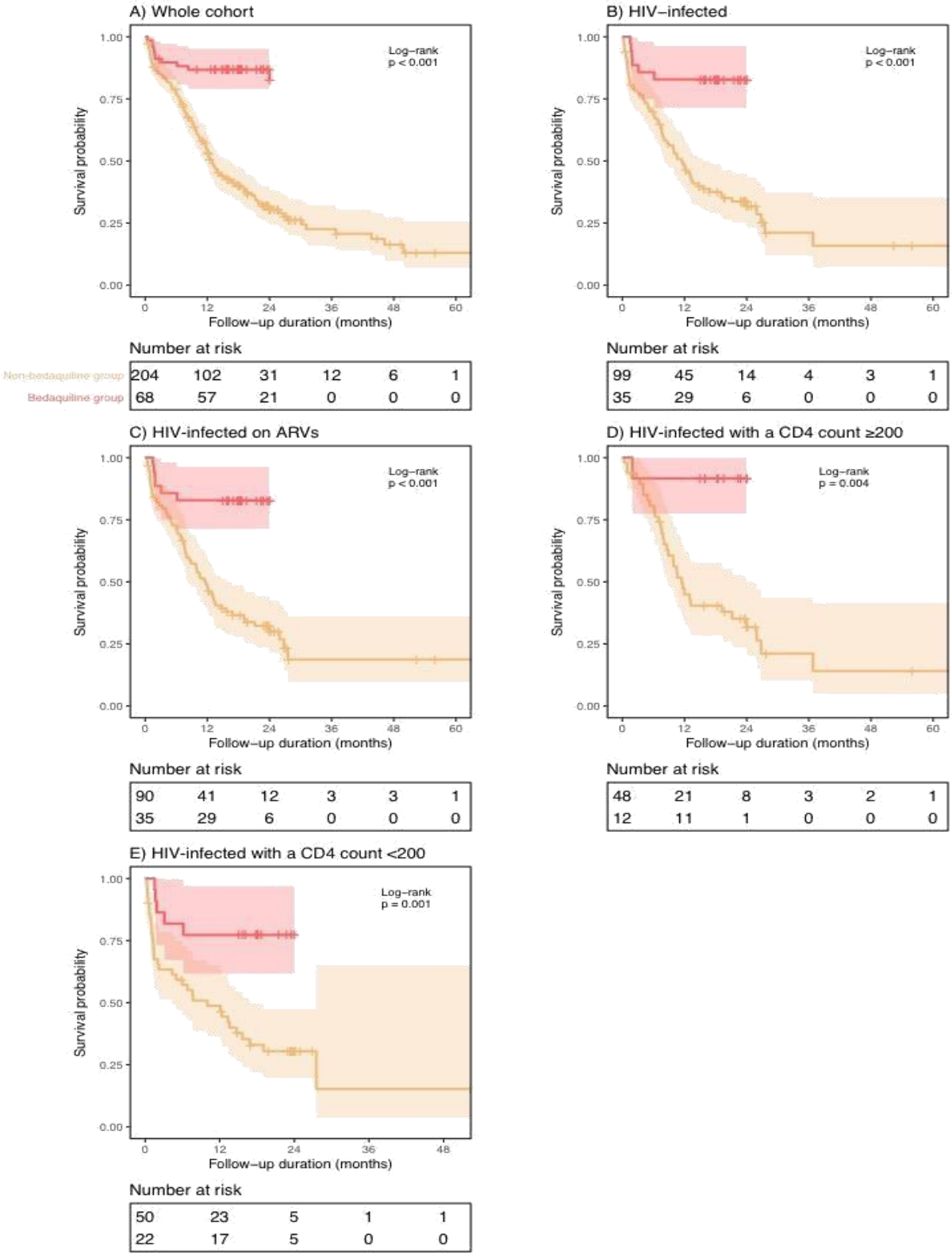

Figure 2: Kaplan-Meier survival estimate for patients in the bedaquiline (Bdq) and the non-bedaquiline (non-Bdq) groups. Shading indicates the 95% confidence interval and plus signs represent patients censoring events. A) Whole cohort. B) HIV-infected patients. C) HIV-infected patients who received ARV. HIV-infected patients whose CD4 count were D) greater than or equal to 200 cells/µl, and E). CD4 count were less than 200 cells/µl.

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Chapter Four: Linezolid interruption in patients with fluoroquinolone-resistant tuberculosis receiving a bedaquiline-based treatment regimen

Abstract

Treatment outcomes of extensively drug-resistant tuberculosis (XDR-TB) patients are sub-optimal and treatment options remain limited. Linezolid is associated with improved outcomes but also substantial toxicity, and details about the relationship between these are lacking from resource-poor HIV-endemic settings.

We prospectively followed up 63 South African XDR-TB patients (58.7% HIV-infected; median CD4 131 cells/μl) between 2014 and 2018. The frequency and severity of linezolid-associated adverse events and the impact on treatment outcomes were compared between linezolid interrupters and non-interrupters.

Twenty-two patients (34.9%) discontinued or underwent dose reduction due to presumed linezolid-associated toxicity. Anaemia (77.3% *versus* 7.3%; p<0.001), peripheral neuropathy (63.6% *versus* 14.6%; p=0.003), and optic neuritis (18.2% *versus* 9.8%; p=0.34) occurred more frequently in linezolid interrupters than in non-interrupters. Anaemia, peripheral neuropathy, and optic neuritis occurred at a median of 5, 18 and 23 weeks, respectively, after treatment initiation. Linezolid interruption was not associated with unfavourable outcomes but was strongly associated with HIV co-infection (aHR 4.831 (1.526- 15.297); p=0.007) and bacterial load (culture days to positivity; aHR=0.824 (0.732- 0.927); p=0.001).

Linezolid-related treatment interruption is common, is strongly associated with HIV co-infection, and system-specific toxicity occurs within predictable time frames. These data inform the clinical management of patients with drug resistant TB.

4.1. Introduction

The increasing prevalence of multidrug resistant tuberculosis (MDR-TB) has become a serious public health problem [1]. MDR-TB is defined as *M. tuberculosis* resistant to isoniazid and rifampicin, the two most important TB drugs. Treatment outcomes for MDR-TB are poor and treatment options are limited. Extensively drug resistant (XDR-TB) is defined as MDR-TB with further resistant to a fluoroquinolone and a second line injectable drug. Although injectables are no longer frontline treatment for MDR-TB, in this manuscript we have retained the term XDR-TB and it was the definition used for the duration of the study. Linezolid, usually together with bedaquiline, is now widely used to treat XDR-TB and fluoroquinolone-resistant TB and is associated with improved culture conversion and survival [2-7]. However, linezolid has substantial toxicity and is associated with significant myelosuppression, peripheral neuropathy and optic neuropathy [3, 4, 8]. Thus, toxicity often leads to interruption of linezolid (stopping the drug for a variable period of time or reducing the dose) in 30 to 60% of patients [4, 8].

However, details about the specific relationship between the duration of linezolid treatment and system-specific toxicity, impact of treatment interruption on outcomes, and effect of HIV co-infection are lacking. Moreover, there are very limited data about linezolid toxicity from TB and HIV endemic settings. To address this knowledge gap, we determined the frequency of linezolid-associated toxicity, the temporal relationship between linezolid initiation and system-specific drug toxicity, and the effect of linezolid interruption (dose reduction or discontinuation) on treatment outcomes of XDR-TB patients receiving a bedaquiline-based regimen.

4.2. Methods

4.2.1. Participants

We prospectively followed up 63 patients with culture-confirmed XDR-TB between April 2014 and April 2018. All patients received a bedaquiline-based treatment regimen containing linezolid as one of the major components. The patients were admitted to Brooklyn Chest Hospital, Cape Town, the XDR-TB treatment centre in the Western Cape province of South Africa. Patients' treatment was directly observed by trained health care workers during hospitalisation and after discharge to outpatient treatment centres. Data were captured by a trained researcher; relevant information obtained included demographics, clinical details, medications received and adverse events. Patients were classified as linezolid interrupters (dose reduction or discontinuation) or non-interrupters, and we performed a comparative analysis of linezolid interrupters and non-interrupters to expressly interrogate whether this interruption adversely impact outcomes, and its potential association with HIV co-infection. Ethical approval was obtained from University of Cape Town human research ethics committee.

4.2.2. Diagnosis and medications received

All the patients had culture isolates with *M. tuberculosis* strains resistant to isoniazid, rifampicin, ofloxacin and a second line injectable anti-TB drug, and met XDR-TB diagnosis criteria [9]. They all received a treatment regimen based on a backbone of linezolid and bedaquiline. Linezolid was administered at 600mg daily for one year and bedaquiline at 400mg daily for two weeks, and then 200 mg three times weekly for 22 weeks. The other drugs common to most of the patients were clofazimine, levofloxacin, pyrazinamide (PZA) and para-amino salicylic acid (PAS).

4.2.3. Adverse events profiling

Adverse events were actively reported by trained health care workers using a standardised case report form and were graded according to the modified American National Institute of Health Common Terminology of Criteria for Adverse Events. Grades 0 means no adverse events; grade 1 means mild adverse event, requiring no intervention; grade 2 means moderate adverse event requiring either changing the dose or frequency of the offending drug, or prescribing another drug to manage the adverse event; grade 3 means severe adverse event, enough to stop the offending drug; grade 4 means life threatening or disabling adverse event; grade 5 means death resulting from the adverse event [10].

4.2.4. Outcomes

Treatment outcomes were assigned according to an adapted version of the 2013 world health organisation definitions and reporting frameworks for TB and, the core research definitions for drug-resistant TB clinical trials recommended by Furin *et al* [11, 12]. Patients were said to have achieved a favourable outcome if they were cured or completed treatment; other treatment outcomes: deceased, lost to follow-up and treatment failure, were considered to be unfavourable.

4.2.5. Statistical analysis

The effect of linezolid interruption was determined by comparative analysis of demographics, clinical characteristics and treatment outcomes. Qualitative and quantitative variables were reported in percentages and median (interquartile range; IQR). Quantitative and qualitative variables were compared using Mann-Whitney U and chi-square or Fisher's exact tests respectively. Univariate cox proportional hazard model was used to estimate the relationship between independent variables (demographic and clinical characteristics), and selected

outcome variables (mortality, the development of linezolid associated adverse events, linezolid interruption, culture conversion and unfavourable outcome). Multivariate models included variables that were significantly associated with outcomes and pre-selected variables. A p-value of <0.05 was taken as statistically significant. Kaplan-Meier curves for the probability of survival was estimated considering the duration between the day of treatment initiation and follow-up censor date. Comparison between strata (HIV-infected vs HIV non-infected, linezolid treatment greater than three months vs linezolid treatment less than three months) was reported as hazard ratio. Statistical analysis was done using SPSS (Version 25).

4.3. Results

4.3.1. Demographic and clinical characteristics

Sixty-three XDR-TB patients met the diagnostic requirements for this study. Demographic and clinical characteristics are reported in Table 1. The median age at admission was 37 (IQR 30-44) years, and 39 (61.9%) were males. Median weight at admission was 51.8 (IQR 46.0-58.6) kg and patients were on admission for a median of 155 (IQR 102-214) days. 37 (58.7%) patients were HIV-infected, the median CD4 count was 131 (56-257) cells/µl at admission, and all were on antiretroviral therapy. Patients received a median of 8 (7-8) anti-TB drugs with linezolid and bedaquiline being the major components. Drugs used in the regimen are outlined in Table 2. Linezolid interruption due to adverse events occurred in 22 (34.9%) patients during the course of treatment while the remaining 41 (65.1%) completed one year of uninterrupted linezolid therapy. Of the 22 patients who had linezolid interruption, 10 had dosage reduction from 600mg to 300mg daily, while 12 had linezolid discontinued.

4.3.2. Adverse events

A total of 208 adverse events were reported by 57 (90.5%) patients; a median of 3 (IQR 2-5) adverse events were reported in the whole cohort. 33 (52.4%), 45 (71.4%) and 36 (57.1%) patients reported grade 1, grade 2 and grade 3 adverse events, respectively. No patients had life-threatening adverse events or died from them. Anaemia (31.7%), peripheral neuropathy (31.7%) and body pains (27%) were the most commonly reported adverse events in the whole cohort. Comparison of adverse events between linezolid interrupters and non-interrupters are outlined in Table 3. Anaemia, peripheral neuropathy and optic neuritis developed a median of 5 (IQR 4-10) weeks, 18 (IQR 11-24) weeks and 23 (IQR 21-26) weeks, after linezolid treatment initiation (Online supplement figure S1). In patients who developed anaemia (haemoglobin level< 10g/dl), 62.5% and 87.5% of them had it within eight and twelve weeks of treatment initiation, respectively, with a median of 26.1% (IQR 10.4-36.1) drop in baseline haemoglobin by 12 weeks of treatment. Table 4 shows the cumulative number of patients that developed adverse events with treatment progression. Anaemia ($p<0.001$) and peripheral neuropathy ($p=0.003$) occurred more frequently in linezolid interrupters. Two of these patients received blood transfusion, and two others had nutritional support.

Although we observed no difference in the proportion of HIV-infected patients (89.2%) who reported at least one adverse event compared to the non-infected patients (88.5%), there were more cases of linezolid interruption in HIV-infected patients (40.5%) compared to the non-infected patients (26.9%). Kaplan-Meier estimate also suggested that HIV-infected patients are more likely to have linezolid interruption within 18 months of treatment (HR1.74; $p=0.23$; Figure 1).

Multivariate analysis showed that duration of linezolid treatment is an independent predictor of linezolid interruption in the whole cohort (HR=0.993; $p<0.001$). It also suggested that HIV-

infected patients (HR=4.831; p=0.007), and patients with higher bacteria load (culture days to positivity; HR=0.824; p=0.001) had higher probability of linezolid interruption (Table 5). Kaplan-Meier survival estimate showed no difference in the probability of unfavourable outcome between linezolid interrupters and non-interrupters (p=0.59; Online supplementary Figure S2), it also showed that patients who received linezolid for greater than three months are more likely to survive (HR=39.9; p<0.001; Figure 1).

4.4. Discussion

This is the first prospective study on probable linezolid associated adverse events in XDR-TB patients from a TB/HIV endemic country. Our major findings were that linezolid interruption is common; the adverse events causing linezolid interruption occur at "predictable" time-points; HIV co-infection and bacterial burden are associated with linezolid interruption and linezolid interruption does not affect treatment outcomes.

Our study established that the use of linezolid in treatment regimen for XDR-TB, as recommended by the WHO is associated with several adverse events, especially peripheral neuropathy and anaemia; this is similar to findings from other studies [13-15]. Over one third of patients had linezolid interruption in their treatment regimen following the development of an adverse event. Adverse events that were likely due to linezolid toxicity occurred within predictable time frames. The predictability of these events can inform patient care and guide physicians and health care workers in patients management, possibly informing dose adjustment at critical time points in a bid to prevent the occurrence or severity of adverse events.

Several methods to reduce linezolid associated adverse events have been proposed. Deliberate reduction in linezolid dosage at specific times in the course of treatment, when adverse events are known to develop may mitigate or outrightly prevent the occurrence of such adverse events

[15, 16]. A shorter treatment regimen has also been proposed , following the preparation of suitable protocol, approval by national ethics committee and delivery under WHO recommended standards [17]. This was corroborated by a study suggesting that linezolid cumulative dose and days of exposure play an important role in the development of adverse event [18]. Therapeutic drug monitoring has been suggested for patients on long-term linezolid treatment, but the cost and the rigours involved make it less feasible; a limited sampling strategy which is cheaper, less time consuming and more feasible has been proposed to individualise linezolid dosing [19, 20]. Recently, a linezolid related adverse events predictive score (LAPS) was developed as a tool for clinicians to assess pre-therapeutic risk of patients to developing those adverse events [21]. LAPS entails assigning scores for certain selected clinical risk factors in patients and grading the summation to predict the development of linezolid associated adverse events.

Yet the effect of linezolid interruption on treatment outcomes remains unclear and has rarely been described in HIV-infected XDR-TB patients from endemic countries. In this study, we explored the relationship between HIV infection and linezolid interruption in patients with drug resistant tuberculosis. We found that HIV co-infection contributed significantly to the occurrence of linezolid interruption. This is in keeping with numerous studies that show higher adverse event rates and consequent drug withdrawal in HIV-infected compared to the un-infected patients [22-24]. HIV infection also contributed to the development of unfavourable outcome, in this study.

Time to sputum culture positivity in patients has been used over the years as a proxy for disease severity [25, 26]. In this study, it correlated significantly with linezolid interruption and this may be an indication that patients who are more sick at the commencement of therapy are more likely to interrupt treatment. Attending physician may be required to monitor them more closely and make individualised dosage plan for such patients.

There were a few limitations to this study. All the patients in this study were hospitalised in the designated treatment centre during the course of treatment, thus, selection bias might have affected the findings. However, the programmatic policy at the centre requires all patients to be hospitalised at least in the intensive phase of therapy. Given the small sample size, the study may not have been sufficiently powered to detect the differences between patients who had linezolid interruption and those who did not. This however is arguably one of the linezolid original studies available with the highest number of participants. This study was conducted in a TB/ HIV endemic setting with a very high enrolment on antiretroviral (ARV) therapy; findings may be different in countries with low HIV prevalence or those with low ARV coverage.

In conclusion, linezolid associated system-specific toxicity occurs within predictable time frames and it is commonly associated with treatment interruption. This prospective study from a TB endemic country demonstrates that linezolid interruption does not negatively impact treatment outcomes though larger studies are needed to confirm this finding. These data inform the use of linezolid for DR-TB treatment in TB endemic countries.

Table 4.1: Demographic, clinical characteristics and treatment outcomes of XDR-TB patients treated with a linezolid-and bedaquiline-based regimen. Data are reflected as number of persons (%) unless otherwise stated.

Variables	**Patients without linezolid interruption (n=41)**	**Patients with linezolid interruption (dose reduction or discontinuation; n=22)**	**p-values**
Gender (Male)	27 (65.9)	12 (54.5)	0.38
Weight (kg)	53.7(IQR 46.5-60.8)	48.7 (IQR 42.9-54.8)	0.17
Age (years)	36 (IQR 29-44)	38 (IQR 31.5-46.3)	0.36
Admission duration (days)	155 (IQR 106-222)	155 (IQR 107-210)	0.71
Duration of linezolid treatment (days)	365 (IQR 181-366)	231.5 (IQR 151-366)	<0.001
HIV-infected	22 (53.7)	15 (68.2)	0.27
CD4 Count/µl	169 (IQR 55-252)	127 (IQR 56-257)	0.37
Patients with previous TB treatment	21 (51.2)	11 (50)	0.93
Number of anti-TB drugs	8 (7-8)	7 (7-8)	0.31
Favourable outcome (Cured/Completed treatment)	30 (73.2)	15 (68.2)	0.68
Unfavourable outcomes	11 (26.8)	7 (31.8)	

Table 4.2: Drugs used in the treatment regimens and the number (%) of patients who received them stratified by linezolid interruption.

Drug	**Patients without linezolid interruption (n=41)**	**Patients with linezolid interruption (dose reduction or discontinuation; n=22)**	**p-values**
Linezolid	41 (100)	22 (100)	*N/A
Bedaquiline	41 (100)	22 (100)	*N/A
Clofazimine	40 (97.6)	22 (100)	0.46
Ethambutol	15 (36.6)	4 (18.2)	0.13
Ethionamide	11 (26.8)	3 (13.6)	0.23
Isoniazid	12 (29.3)	8 (36.4)	0.56
Levofloxacin	40 (97.6)	21 (95.5)	0.65
Para-aminosalicylic acid	39 (95.1)	21 (95.5)	0.95
Pyrazinamide	40 (97.6)	21 (95.5)	0.65
Terizidone	39 (95.1)	20 (90.9)	0.51
Moxifloxacin	8 (19.5)	2 (9.1)	0.28
Delamanid	5 (12.2)	3 (13.6)	0.87

*N/A= Not applicable

Table 4.3: Number (%) of patients experiencing adverse events depending on linezolid interruption.

Variables	**Patients without linezolid interruption (n=41)**	**Patients with linezolid interruption (dose reduction or discontinuation; n=22)**	**p-values**
Peripheral neuropathy	6 (14.6)	14 (63.6)	0.003
Anaemia	3 (7.3)	17 (77.3)	<0.001
Arthralgia	6 (14.6)	5 (22.7)	0.42
Skin reaction	8 (19.5)	8 (36.4)	0.14
Body pains	11 (26.8)	6 (27.3)	0.97
Optic neuritis	4 (9.8)	4 (18.2)	0.34
Dizziness	5 (12.2)	5 (22.7)	0.28
Dyspepsia	2 (4.9)	1 (4.5)	0.95
Nausea	4 (9.8)	5 (22.7)	0.16
Vomiting	7 (17.1)	5 (22.7)	0.59
Epigastric pain	6 (14.6)	5 (22.7)	0.42
Diarrhoea	4 (9.8)	2 (9.1)	0.93
Thyroid dysfunction	3 (7.3)	4 (18.2)	0.19
Psychosis	3 (7.3)	2 (9.1)	0.81

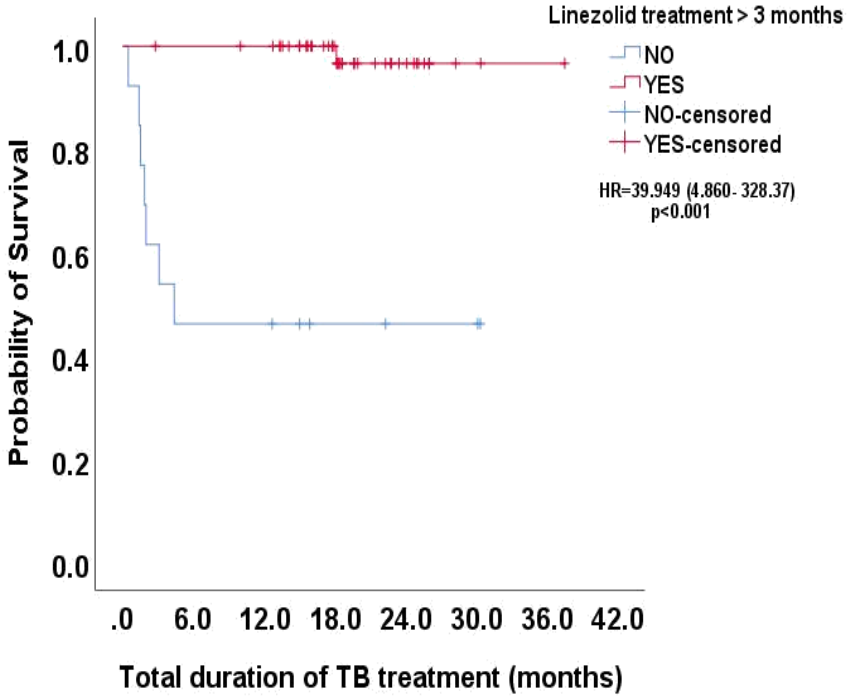
Table 4.4: Cumulative number (%) of patients that experienced an adverse event (types) with increased treatment duration.

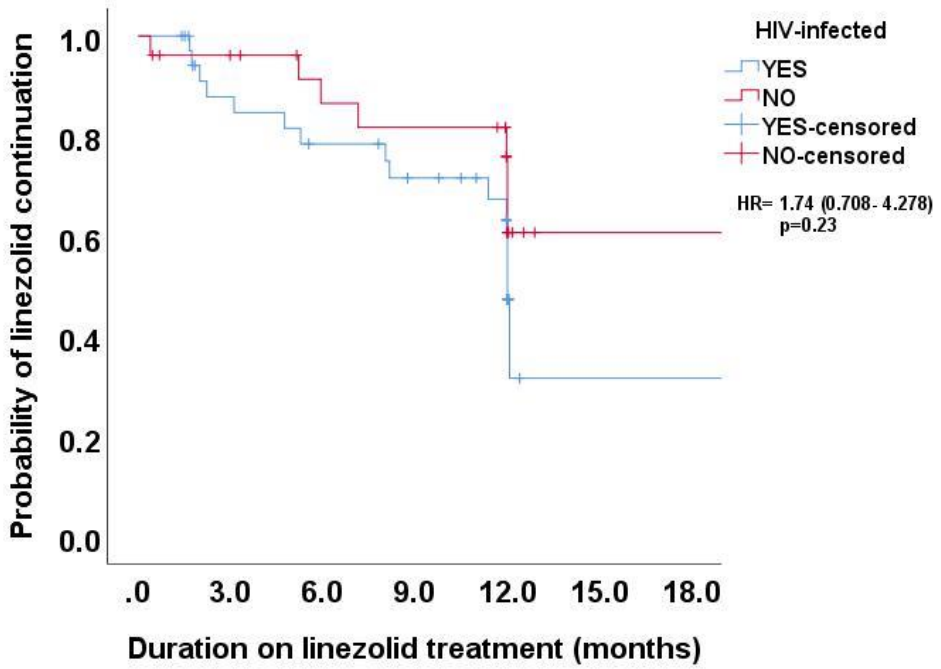
Treatment Duration	**Patients that developed any adverse event (n=22)**	**Patients that developed anaemia (n=16)**	**Patients that developed peripheral neuropathy (n=13)**	**Patients that developed optic neuritis (n=4)**
1 Month	5 (22.7)	5 (31.3)	0 (0)	0 (0)
2 Months	10 (45.5)	10 (62.5)	2 (15.4)	0 (0)
3 Months	15 (68.2)	14 (87.5)	6 (46.2)	0 (0)
4 Months	17 (77.3)	16 (100)	6 (46.2)	0 (0)
5 Months	18 (81.8)	16 (100)	7 (53.8)	1 (25)
6 Months	21 (95.5)	16 (100)	10 (76.9)	3 (75)
9 months	22 (100)	16 (100)	12 (92.3)	4 (100)

Table 4.5: Univariate and multivariate cox proportional hazard model interrogating factors associated with unfavourable outcome and linezolid interruption.

	Unfavourable outcome (n=18)		Linezolid interruption (n=22)	
Univariate analysis				
Variable	Hazard Ratio (95% C.I.)	p-value	Hazard Ratio (95% C.I.)	p-value
Weight(kg)	0.977 (0.934- 1.023)	0.32	0.973 (0.933-1.013)	0.19
Gender (male)	2.655 (1.024- 6.889)	0.05	1.790 (0.758-4.229)	0.18
Days hospitalized	0.990 (0.982- 0.997)	0.008	0.998 (0.994-1.002)	0.38
HIV-infected	1.763 (0.647-4.806)	0.27	1.901 (0.768- 4.779)	0.17
Age (years)	1.011 (0.964- 1.060)	0.65	1.025 (0.981-1.071)	0.28
Previous tuberculosis treatment	1.170 (0.706- 2.109)	0.51	1.096 (0.719-1.671)	0.67
Levofloxacin/Moxifloxacin treatment	0.045 (0.00- 1266)	0.55	1.162 (0.151- 8.908)	0.89
PZA treatment	3.715 (0.455- 30.357)	0.666	2.143 (0.283-16.261)	0.46
Number of TB drugs	0.975 (0.637- 1.492)	0.91	0.898 (0.591-1.366)	0.62
Smear grade (baseline)	2.064 (0.902- 4.722)	0.09	1.287 (0.762- 2.173)	0.35
[#]Time to culture positivity in days	0.954 (0.890- 1.023)	0.19	0.886 (0.818- 0.961)	0.003
Duration on linezolid (days)	0.995 (0.992- 0.998)	0.03	0.997 (0.994-1.000)	0.02
Multivariate analysis				
Weight (kg)	1.015 (0.967- 1.066)	0.55	0.975 (0.929- 1.024)	0.32
Gender (male)	1.411 (0.473- 4.207)	0.54	1.469 (0.513- 4.210)	0.47
Duration on linezolid	0.996 (0.991- 1.000)	0.05	0.993 (0.989- 0.997)	<0.001
HIV-infected	2.211 (0.645- 7.575)	0.21	4.831 (1.526- 15.29)	0.007
Days hospitalized	0.996 (0.987- 1.005)	0.36	N/A*	N/A*
Linezolid interruption	0.981 (0.351- 2.744)	0.97	N/A*	N/A*
[#]Time to culture positivity in days	N/A*	N/A*	0.824 (0.732- 0.927)	0.001
Age	N/A*	N/A*	1.093 (1.030- 1.160)	0.003

N/A*=Not applicable, [#]Baseline sputum sample was used





Number at risk

YES	50	50	50	30	12	2	1	1
NO	13	6	6	3	2	2	0	0

Number at risk

YES	15	11	8	6	4	0	0
NO	7	6	4	3	2	0	0

Figure 1 (A): Kaplan-Meier survival estimate for patients who received linezolid for more than 3 months in their treatment regimen and (B) for the probability of linezolid continuation in HIV-infected patient during an 18 months treatment period

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Chapter 5: A regimen containing bedaquiline and delamanid compared to bedaquiline in patients with drug resistant tuberculosis

Abstract

There are limited data about combining delamanid and bedaquiline in drug-resistant tuberculosis (DR-TB) regimens. Prospective long-term outcome data, including in HIV-infected persons, are unavailable.

We prospectively followed up 122 South Africans (52.5% HIV-infected) with DR-TB and poor prognostic features between 2014 and 2018. We compared outcomes and safety in those who received a bedaquiline-based regimen (n=82) to those who received a bedaquiline-delamanid combination regimen (n=40).

There was no significant difference in 6-month culture conversion (92.5% versus 81.8%; p=0.26) and 18-month favourable outcome rate (63.4% versus 67.5%; p=0.66) in the bedaquiline versus the bedaquiline-delamanid combination group, despite the latter having more advanced drug resistance (3.7% versus 22.5% resistant > 5 drugs; p= 0.001) and higher pre-treatment failure rates (12.2% versus 52.5% with pre-treatment MDR-TB therapy failure; p <0.001). Although the proportion of QTcF prolongation was higher in the combination group [>60 ms from baseline (p=0.001) or > 450 ms during treatment (p=0.001)], there were no symptomatic cases or drug withdrawal in either group. Results were similar in HIV-infected patients.

A bedaquiline-delamanid combination regimen showed comparable long-term safety, to a bedaquiline-based regimen, in patients with DR-TB irrespective of HIV status. These data inform regimen selection in patients with DR-TB from TB endemic settings.

5.1. Introduction

Drug resistant tuberculosis (DR-TB) constitutes a threat to TB control globally. In 2017, and despite high rates of underreporting and underdiagnosis, there was a 5% increase in the absolute number of DR-TB cases detected compared to 2016 [1, 2]. Treatment outcomes of MDR-TB have also remained poor with a treatment success rate of 55% reported in 2017 [3, 4]. However, this is expected to improve given that newer and repurposed drugs have now been recommended by the world health organisation (WHO) for the treatment of multidrug resistant TB (MDR-TB) [5]. Indeed, studies and regimens containing bedaquiline and linezolid have shown impressive efficacy in clinical trials and real-life programmatic use [6-10].

Nevertheless, even with the use of these newer drugs, successful outcomes and treatment options are limited in patients with fluoroquinolone-resistant and XDR-TB [7]. Thus, there is frequently a difficulty in constituting an effective treatment regimen [11-15]. Patients who have had previous exposure to second line TB medication, developed resistance to multiple drugs, developed intolerability due to adverse events [16], and those who have failed therapy on bedaquiline-based regimens, are all predisposed to having a poor prognosis. In this group of patients, it is challenging to constitute a treatment regimen containing at least 4 to 5 likely effective drugs (drugs to which the isolate was susceptible and/ or to which patients had less than one month or no previous exposure) without simultaneously including bedaquiline and delamanid. The latter, a group C drug, remains widely used in clinical practice although there is currently limited evidence about the efficacy of delamanid for the treatment of MDR-TB [17-19].

Thus, in appropriate patients with high level resistance and/ or poor prognosis there is an increasing need to use a bedaquiline-delamanid combination. However, the potential for synergistic QTc prolongation from both drugs, predisposing patients to cardiac arrythmias and

sudden death has created unease over their inclusion in the same treatment regimen [4, 20-22]. Despite this concern and more widespread use, few studies have reported on the concurrent use of delamanid and bedaquiline and have mostly described early safety and efficacy of the combination in retrospective cohorts [23, 24]. There are, however, no prospective and long-term data addressing safety, and none addressing efficacy of the combination regimen in patients with poor prognosis from a TB endemic setting. Furthermore, there are limited data about use of the bedaquiline-delamanid combination in HIV co-infected patients, and those with high level resistance e.g. patients with XDR-TB. Another major limitation has been the lack of comparative data from a bedaquiline only control group, so that the net effect of delamanid safety (and QT prolongation) over that of bedaquiline, could be ascertained. We therefore compared the treatment outcomes and adverse event profiles of DR-TB patients with poor prognostic features on a bedaquiline-based regimen to those who had received the bedaquiline-delamanid combination.

5.2. Methods

5.2.1. Study design and participants

We prospectively recruited, through an ongoing clinical registry, microbiologically confirmed MDR-TB patients who were admitted to Brooklyn Chest Hospital, the designated treatment centre for drug resistant TB in the Western Cape province in South Africa. However, a per protocol analysis plan was only formulated retrospectively. All patients were admitted between January 2014 and April 2018. Patients were included in the study if their drug susceptibility and testing done prior to recruitment showed resistance to at least rifampicin and isoniazid. Sputum drug susceptibility and testing was repeated on a monthly basis following treatment initiation, to monitor treatment progress. Each patient received either a bedaquiline-based or a bedaquiline-delamanid combination regimen. Medications were administered by trained health

care workers while patients were on admission and on outpatient basis after discharged from the hospital.

Adverse events were reported by a medically qualified health care worker, using a pharmacovigilance report form provided for each patient. Each patient had an electrocardiogram (ECG) done before treatment initiation and at least on a monthly basis afterwards. QT interval was corrected using Friderica's formula (QTcF), and values greater than 450ms qualifies a patient for closer review by attending physician. Patients' demographic and clinical data were captured by a trained researcher, laboratory reports were regularly updated on a dedicated database using a standard case report form. A written consent was obtained from every participant, and ethical clearance was obtained from the University of Cape Town Human Research Ethics Committee.

5.2.2. Treatment groups

All patients were assigned a treatment group based on the drugs that constitute the backbone of their regimen. Bedaquiline was the backbone in the bedaquiline-based regimen group while the second group received a regimen whose backbone was a combination of bedaquiline and delamanid (bedaquiline-delamanid), administered concurrently. Indications for receiving the combination therapy were inability to construct an effective regimen (at least 4 likely effective drugs) due to extensive drug resistance patterns or adverse events, strengthening of a regimen due to late conversion, or extensive lung disease and patients who have previously failed on a bedaquiline-based regimen. Medications were individualised for patients based on their phenotypic drug susceptibility testing results.

5.2.3. Outcomes

Culture conversion was defined as two consecutive negative sputum culture results, taken at least 30 days apart (one missing or contaminated culture was allowed between negative

cultures, and inability to produce sputum was considered to be a negative result). Culture conversion status was compared between the two groups at two months, six months and twelve months following treatment initiation. In the first six months of therapy, changes in the QTcF interval from baseline values were also compared between the two groups to establish how it is impacted by the treatment regimens. At follow-up censor date which was a minimum of 18 months, patients were assigned to have had a favourable outcome if they completed treatment or were cured, those who died during treatment, failed to achieve culture conversion or lost to follow-up were said to have had an unfavourable outcome.

5.2.4. Statistical Analysis

The impact of delamanid was determined by comparative analysis of demographics, clinical characteristics, and treatment outcomes. Qualitative and quantitative variables were reported in percentages and median (interquartile range; IQR). Quantitative and qualitative variables were compared using Mann-Whitney U and chi-square or Fisher's exact tests respectively. A univariate Cox proportional hazard model was used to estimate the relationship between independent variables (demographic and clinical characteristics) and the development of unfavourable outcome and, having at least one QTc value of greater than 450 ms; variables with a p-value less than 0.3 were included in the multivariate model. A p-value of <0.05 was taken as statistically significant. Kaplan-Meier curves for the probability of achieving an unfavourable outcome and the proportion of patients with culture positivity were estimated considering the duration between the day of treatment initiation and follow-up censor date. Comparison between strata (bedaquiline-based therapy and bedaquiline-delamanid combination therapy) were undertaken using a log-rank test. Statistical analysis was done using IBM SPSS Statistics Version 25.0(IBM Corp., Armonk, NY, USA).

5.3. Results

5.3.1. Demographic and clinical characteristics

In this study, a total of 122 culture-confirmed multidrug-resistant TB patients were enrolled into either a bedaquiline-based regimen group or a bedaquiline-delamanid combination regimen group. Median age at admission was 34 (IQR 27-42) years, they were on admission for a median 161 (IQR 102-230) days and 74 (60.7%) were male. The median weight at admission was 51.8 (IQR 43.8-59.0) kg, 64 (52.5%) patients were HIV-infected with median CD4 count of 154 (IQR 57- 332) cells/µl and they were all on antiretroviral therapy. Isolates from 11 (9%) patients were outrightly multidrug resistant (MDR-TB), 25 (20.5%) patients had further resistance to either a fluoroquinolone or a second line injectable (Pre-XDR TB), while 86 (70.5%) patients were resistant to both (XDR-TB).

There were 82 (67.2%) patients in the group who received bedaquiline-based regimen, they were hospitalised for a median 155 (IQR93-210) days. Patients demographic and clinical characteristics are outlined in Table 1. They received a median 8 (IQR 7-9) medications in the regimen which essentially comprised of bedaquiline, clofazimine, levofloxacin and linezolid as the major components. Other drugs and the proportion of patients who received them are outlined in Table 2. All patients received clofazimine or a fluoroquinolone which are known QTc prolonging drugs. 23 patients (28.1%) received both drugs, 55 patients (67.1%) received only clofazimine while 3 (3.7%) patients received only moxifloxacin in their regimen.

In the bedaquiline-delamanid combination regimen group, 40 (32.8%) patients were enrolled and they were hospitalized for a median 204 (IQR 124-295) days. 29 (72.5%) patients have been previously treated for TB, 9 (22.5%) were resistant to at least five drugs; other markers of disease severity are highlighted in Table 1. They received a median 10 (IQR 8-11) medications in the regimen which was significantly more than medications received in the

bedaquiline-based regimen. The major medications in this regimen are delamanid, bedaquiline, clofazimine, levofloxacin and linezolid. 37 (92.5%) patients in this group received at least one of clofazimine and moxifloxacin. 14 patients (35.0%) received both drugs, 21 patients (52.5%) received only clofazimine while 2 (5.0%) patients received only moxifloxacin in their regimen.

5.3.2. Markers of disease severity

There were significantly more patients (72.5%) in the bedaquiline-delamanid combination regimen group with previous exposure to TB treatment compared to those in the bedaquiline-based regimen (48.8%; p=0.01). More patients in the combination therapy group have previously failed TB treatment compared to those in the bedaquiline group (52.5% vs 12.2%; p<0001). The bedaquiline-delamanid combination regimen group also had significantly more patients with resistance to more than five drugs (22.5% vs 3.7%; p=0001). Other markers of disease severity including HIV-infection, microbial burden and weight less than 50 kg at admission were mostly higher in the bedaquiline-delamanid combination regimen group (Table 1).

5.3.3. Culture conversion

In the bedaquiline-based regimen group, 52 patients (63.4%) were culture positive at recruitment, 92.5% of those with laboratory results had achieved culture conversion by six months of treatment. Of the 42 HIV-infected patients in this group, 23 (54.8) were culture positive at recruitment, and 93.8% of those with laboratory results have achieved culture conversion by six months of treatment.

In the bedaquiline-delamanid combination regimen, 26 patients (65%) were culture positive at recruitment, 81.8% of those with laboratory results had achieved culture conversion by six months of treatment. Of the 22 HIV-infected patients in this group, 13 (59.1%) were culture positive at recruitment, 83.3% of those with laboratory results had achieved culture conversion

by six months of treatment. Comparison of culture conversion rates in both groups are outlined in Table S1 (Online supplement). In time to event analysis, there were more patients in the bedaquiline-delamanid combination group with culture positivity compared to those in the bedaquiline group by the end of the fifteenth month (p=0.04; Figure 1).

5.3.4. Efficacy of the treatment regimens

In the bedaquiline-based regimen group, 52 patients (63.4%) achieved a favourable outcome while the remaining patients had unfavourable outcome by the end of follow-up period. 29 (69.1%) out of 42 HIV-infected patients in this group also, achieved a favourable outcome. In the bedaquiline-delamanid combination regimen group, 27 patients (67.5%) achieved a favourable outcome while the remaining patients had unfavourable outcome by the end of follow-up period. 15 (68.2%) out of 22 HIV-infected patients in this group also, achieved a favourable outcome. There was no significant difference in the favourable outcome rate between the two groups even when they were stratified by resistance patterns (Table 1; online supplement Table S2). In time to event analysis, there was no difference in the probability of achieving an unfavourable outcome between the two groups (p=0.54; Figure 1). Regression analysis showed that moxifloxacin (HR 1.023; p=0.89) and clofazimine (HR 0.711; p=0.35) which were the other QTcF prolonging drugs used in both regimens did not predispose to having unfavourable outcome (Table 3). It also suggested that days to sputum culture positivity less than seven days (H.R.= 2.712; p=0.006) and resistance to more than five drugs (H.R.= 2.173; p=0.08) are independent predictors of an unfavourable outcome (Table 3).

5.3.5. Adverse events

In the bedaquiline-based regimen group, 73 patients (89.0%) reported a total of 250 adverse events, each patient reporting a median 2 (IQR 1-4) adverse events in the course of treatment. The most commonly reported adverse events were hearing loss (50.0%), most likely from

previous second line injectable treatment, elevated liver enzymes (28%) with median ALT of 112 U/L (IQR 81-173) in affected patients, anaemia (34.1%), peripheral neuropathy (22.0%) and vomiting (24.4%). ALT elevation in the HIV-infected patients in this group was by a median 107 U/L (IQR 71-154).

In the bedaquiline-delamanid combination regimen group, 37 patients (92.5%) reported a total of 125 adverse events, each patient reporting a median 3 (IQR 2-4) adverse events in the course of treatment. The most commonly reported adverse events in this group were hearing loss (45%), elevated liver enzymes (32.5%) with median ALT of 111U/L (IQR 85-155), anaemia (37.5%) and peripheral neuropathy (30.0%). ALT elevation in the HIV-infected patients in this group was by a median 133 U/L (IQR 91-155), this was essentially similar to those in the bedaquiline regimen (p=0.34). There were no significant differences in the occurrence of adverse events reported in the two groups except for psychosis which was likely associated with higher rate of simultaneous use of terizidone and high dose isoniazid in the bedaquiline-delamanid combination regimen group. Other adverse events and the proportion of patients who had them are reported in Table 4.

5.3.6. QTcF interval changes

The median baseline QTcF value for the bedaquiline-based regimen group was 408 ms (IQR 388-425). In the first six months of treatment, there was a maximum QTcF prolongation of median 27 ms (IQR 13-42) from the baseline values, and only 6 patients (7.3%) had a QTcF prolongation greater than 60 ms from baseline values. 16 patients (19.5%) in this group also had at least one QTcF value greater than 450 ms in the course of treatment, but none reached the threshold limit of 500 ms, and none of them had bedaquiline discontinued due to changes in QTcF values (Table 5).

The median baseline QTcF value for the bedaquiline-delamanid regimen group was 419 ms (389-436). In the first six months of treatment, there was a maximum QTcF prolongation of median 23 ms (8-54) from the baseline values, and only 7 patients (20.6%) had a QTcF prolongation greater than 60 ms from baseline values. 15 patients (44.1%) in this group also had at least one QTcF value greater than 450 ms in the course of treatment, none of them reached the threshold limit of 500 ms, and neither bedaquiline nor delamanid was discontinued in any of the patients due to changes in QTcF values (Table 5).

There was no definitive pattern to the changes (increase or decrease) in QTcF values observe over a period of 6 months (online supplement Figure S1, but there were more patients in the bedaquiline-delamanid regimen group who reported a QTcF prolongation of more than 60 ms from baseline values ($p<0.001$) and at least one QTcF value greater than 450 ms ($p<0.001$) in the course of treatment. Increasing age (H.R.=1.039; p= 0.04) and the use of delamanid (H.R.= 3.504; p= 003) were independent predictors of having at least one QTcF value greater than 450 ms (Table 6). There were however no cardiac symptoms necessitating the withdrawal of delamanid from the treatment regimen from any of the affected patients.

5.4. Discussion

This is the first prospective study comparing long-term treatment outcomes and safety data in drug-resistant TB patients who received bedaquiline versus bedaquiline-delamanid combination therapy. The main findings of the study were that (i) combination therapy was associated with significant QTcF prolongation from baseline values but there were no patients who became symptomatic from a cardiovascular point of view (syncope, collapse, arrythmia, hypotension etc.) or reached the threshold limit of 500ms that would have necessitated recommended withdrawal of either bedaquiline or delamanid (and despite the concomitant use of other QT-prolonging drugs), (ii) bedaquiline-delamanid combination therapy was associated

with modestly good culture conversion and favourable outcome rates (and comparable to a bedaquiline-based regimen) despite being used in patients from a poorer prognostic category i.e. high proportion of patients who had previously been declared therapeutically destitute, and/ or whose isolates were resistant to > 5 drugs, and (iii) these findings were broadly similar in HIV-infected patients.

Thus, our data support the use of the combination in patients in whom there is a difficulty in constituting a regimen with at least four effective drugs. Our data are concordant with 2 recent publications, which also confirmed the safety profile of the bedaquiline/delamanid combination [24, 25]. However, there are several important strengths and incremental contributions of our study findings. Here, we are able to confirm the long-term safety, safety in HIV-infected persons (which has always remained the concern given their higher frequency of adverse events to drugs in general), and importantly we were able to discern the incremental QT prolongation effect of delamanid within the combination, when compared to a bedaquiline only group. This should allay anxiety amongst clinicians who often need to include delamanid in treatment regimens but are concerned by the WHO Guidelines which express reservation about the safety of simultaneous use of these drugs [26]. Other QT-prolonging drugs like clofazimine and moxifloxacin were used substantially, and to a similar extent, in both groups and thus we could account for their effect when comparisons were made. Nevertheless, ECG monitoring is still required when the combination is used, especially together with other QT-prolonging drugs, given that discontinuation of drugs have been documented in a few patients [26]. However, our findings support the notion that this is not a major issue and is uncommon.

We were also able to evaluate the long-term efficacy, in terms of outcomes, in the bedaquiline only and combination groups, respectively. It is reassuring that we found similar long-term outcomes in the bedaquiline-delamanid combination group despite this group having a higher frequency of poor prognostic features (at least half the group were MDR treatment failures

compared to ~10% in the bedaquiline group, and almost a quarter of isolates in the combination group were resistant to 5 or more drugs compared to about ~5% in the bedaquiline only group). Furthermore, usage of WHO group A and group B drugs, i.e. (linezolid, any fluoroquinolone, clofazimine, and terizidone) were similar in both groups. Despite recent findings suggesting limited efficacy of this drug (and when using 6-month culture conversion as an outcome) [17], our data suggest that delamanid may be a useful addition in patients where an appropriate regimen of 4 to 5 likely effective drugs cannot be constituted because of toxicity or high-level resistance. Indeed, despite the combination group having an ~50% prior treatment failure rate, the 6-month culture conversion rate in this group was over 80%, and the overall long-term favourable outcome rate was almost 70%. This far exceeds the dismal outcomes seen with XDR-TB prior to the advent of newer drugs [27, 28]. Nevertheless, ~10% of patients in each group failed treatment. Thus, programmatically incurable TB is an emerging problem in TB endemic countries and public health efforts are needed to manage such patients on a long-term basis. Besides establishing palliative care and long-term community-based residential facilities [29], preventative measures such as optimal antibiotic stewardship, active case finding, and wider roll-out of new diagnostics and drugs are urgently required [15].

In HIV-infected patients the adverse event profile and treatment outcomes showed similar patterns compared to HIV-uninfected patients. These findings are highly relevant to high TB and HIV-endemic settings. Besides QT-prolongation, other important adverse events, such as elevation of liver enzymes, were found to be similar in the HIV-infected patients in both groups, further confirming safety and compatibility with antiretrovirals in this group.

There are several limitations of our findings. Our study was of limited sample size and a larger study may have shown different results given that cardiovascular events related to QT prolongation is rare. A larger sample size may have also allowed some clear-cut outcome effect to be discerned. However, this study has reported the largest number of patients on bedaquiline-

delamanid combination therapy (n = 40), the largest number of patients with XDR-TB (n = 86), and the multivariable analysis, even when taking into account the poorer prognostic features in the combination group, failed to identify the combination as an independent predictor of outcome. Rather, bacterial load remained the only significant and independent predictor of outcome. This highlights another limitation, which is the failure to evaluate radiographic disease extent at the time of diagnosis, which would better enable us to account for initial disease severity. However, logistical and technical issues prevented us from accessing the pre-treatment chest radiographs. Nevertheless, we were able to get a fairly good comparative estimation of disease severity in both groups through evaluation of other prognostic features such as admission weight, HIV status, CD4 count, previous treatment history, resistance to 5 or more drugs, and mycobacterial load, which are all proxies of disease severity. Selection bias could have also impacted our findings including the cases and controls study design. However, all the patients were prospectively recruited in the same region and over a similar timeframe. It is possible that some patients with events could have been missed as the programme in the Western Cape became more decentralised. However, our recruitment network spanned the entire region and also utilised a region-wide electronic capture and surveillance system.

In summary, our findings suggest that a bedaquiline-delamanid combination has a comparable long-term safety profile to a bedaquiline-based regimen in patients with drug-resistant TB irrespective of HIV status. Delamanid appeared to be a useful adjunct in the treatment of patients with poor prognostic features or high-level resistance where constituting an appropriate regimen would be otherwise challenging. These data inform regimen selection in patients with drug-resistant TB from TB endemic settings.

Table 5.1: Comparison of demographic and clinical characteristics between patients in the bedaquiline and the combination regimen groups. Data are reflected as number of persons (%) or median (interquartile range) unless otherwise stated.

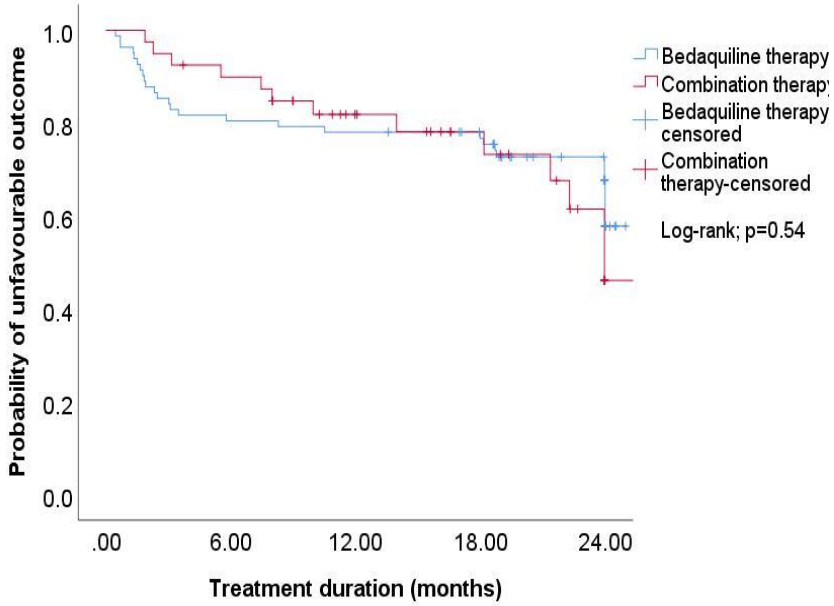
Variables	Patients who received bedaquiline-based regimen (n=82)	Patients who received bedaquiline-delamanid combination regimen (n=40)	p-value
Age (years)	33 (IQR 28-42)	34 (IQR 27-42)	0.04
Gender (male)	50 (61.0)	24 (60.0)	0.92
Weight at admission (kg)	51.8 (IQR 45.6- 58.3)	51.8 (IQR 43.3- 60.8)	0.36
Days hospitalized	155 (IQR 93-210)	204 (IQR 124- 295)	0.38
Number of medications	8 (IQR 7-9)	10 (IQR 8-11)	0.001
5 likely effective medications	59 (72%)	26 (65%)	0.43
Number of adverse events	2 (IQR 1-4)	3 (IQR 2-4)	0.51
Time to culture positivity (days)	14 (IQR 10-17)	10 (IQR 8-14)	0.46
Diagnosis: XDR-TB	67 (81.7)	19 (47.5)	<0.001
PRE-XDR TB	10 (12.2)	15 (37.5)	<0.001
MDR-TB	5 (6.1)	6 (15)	<0.001
Markers of disease severity			
Patients with weight <50kg	34 (41.5)	19 (47.5)	0.53
Patients with previous MDR-TB treatment failure	10 (12.2)	21 (52.5)	<0.001
Patients with previous TB treatment	40 (48.8)	29 (72.5)	0.01
HIV-infected	42 (51.2)	22 (55.0)	0.69
CD4 Count (cells/µl)	135 (60-279)	234 (52- 367)	0.41
Patients with CD4<200 cells/µl	26 (31.7)	10 (29.4)	0.19
Diabetic patient	2 (2.4)	1 (2.5)	0.98
Patients resistant to ≥5 drugs	3 (3.7)	9 (22.5)	0.001
Patients with either resistant to ≥ 5 drugs or previous treatment failure	13 (15.9)	23(57.5)	<0.001
Smear grade>2 plusses	14 (17.1)	8 (20.0)	0.69
Time to culture positivity≤ 7 days	9 (10.9)	7 (17.5)	0.32
Treatment outcome			
Favourable outcome	52 (63.4)	27 (67.5)	0.66
Unfavourable Outcome	30 (36.6)	13 (32.5)	

Table 5.2: List of drugs used in the bedaquiline-based regimen and bedaquiline-delamanid combination regimen and the proportion of patients who received them. Data is n (%).

Drugs	Patients who received bedaquiline-based regimen (n=82)	Patients who received bedaquiline-delamanid combination regimen (n=40)	p-value
Kanamycin	16 (19.5)	8 (20)	0.95
Terizidone	75 (91.5)	33 (82.5)	0.15
Pyrazinamde	80 (97.6)	37 (92.5)	0.19
Para-aminosalicylic acid	75 (91.5)	31 (77.5)	0.03
Meropenem	0 (0)	5 (12.5)	N/A*
Any fluoroquinilone	81 (98.8)	37 (92.5)	0.07
Moxifloxacin	26 (31.7)	16 (40)	0.37
Levofloxacin	81 (98.8)	33 (82.5)	0.001
Linezolid	67 (81.7)	36 (90)	0.24
High dose Isoniazid	34 (41.5)	20 (50)	0.37
Ethionamide	27 (32.9)	13 (32.5)	0.96
Ethambutol	38 (46.3)	14 (35)	0.23
Clofazimine	78 (95.1)	35 (87.5)	0.13
Capreomycin	7 (8.5)	3 (7.5)	0.85
Bedaquiline	82 (100)	40 (100)	N/A*
Delamanid	0 (0)	40 (100)	N/A*

N/A*: Not applicable

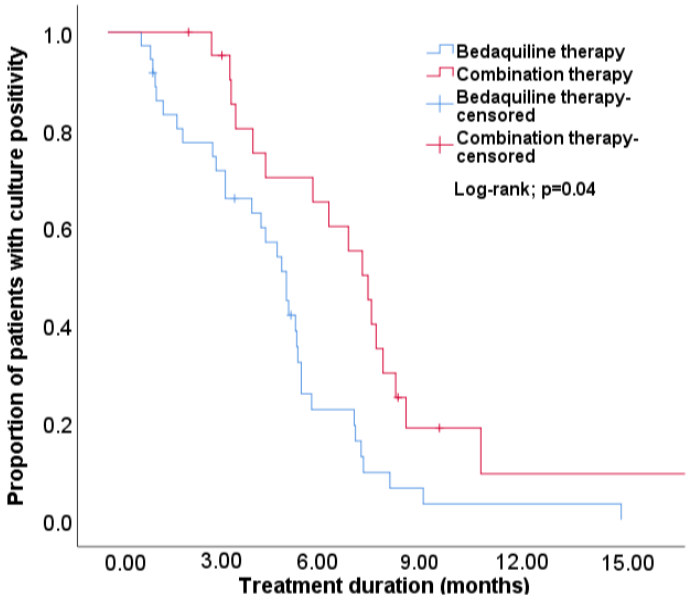
I



Number at risk

B	82	14	12	11	4
DB	40	9	6	5	0

II



Number at risk

B	52	46	42	41	40	40
DB	26	25	24	21	16	16

Figure 1: Kaplan Meier estimate for the probability of achieving an unfavourable outcome (I) and the proportion of patients with culture positivity by the fifteenth month (II) in patients who received bedaquiline-based regimen (B) versus a bedaquiline-delamanid combination regimen (DB).

Table 5.3: Univariate and multivariate Cox proportional hazard model for developing unfavourable outcome in the whole cohort (N=122)

Variables	Hazard ratio (95% C.I)	p-value
Univariate analysis		
Age (years)	0.997 (0.967- 1.028)	0.85
Gender (male)	1.231 (0.662-2.289)	0.51
Weight (kg)	0.986 (0.960- 1.014)	0.33
Age at admission < 50 years	1.137 (0.624- 2.070)	0.68
HIV-infection	1.181 (0.874- 1.595)	0.28
CD4 Count (cells/µl)	1.000 (0.998- 1.002)	0.84
Previous TB treatment	1.013 (0.556- 1.848)	0.97
Previous treatment failures	1.367 (0.580- 3.223)	0.48
Days of admission	1.000 (0.997- 1.002)	0.87
Clofazimine treatment	0.505 (0.122- 2.090)	0.35
Delamanid treatment	0.877 (0.627- 1.225)	0.44
Moxifloxacin treatment	1.023 (0.743- 1.408)	0.89
Levofloxacin treatment	0.968 (0.473- 1.980)	0.93
Any fluoroquinolone	0.897 (0.123- 6.555)	0.92
Linezolid treatment	0.959 (0.426- 2.157)	0.92
Bedaquiline-delamanid treatment	0.814 (0.416- 1.593)	0.55
Number of medications	1.112 (0.935- 1.322)	0.23
Number of adverse events	1.026 (0.898- 1.171)	0.71
5 likely effective drugs	0.840 (0.589- 1.196)	0.33
Resistant to >5 drugs	2.173 (0.900- 5.246)	0.08
TTP* < 7 days	2.712 (1.331- 5.522)	0.006
Smear grade	1.583 (0.779- 3.216)	0.20
Multivariate Analysis		
HIV-infection	1.940 (0.791- 2.751)	0.22
Isolate resistant to >5 drugs	1.940 (0.787- 4.779)	0.15
TTP* < 7 days	2.681 (1.196- 6.011)	0.02*
Number of medications	1.144 (0.957- 1.368)	0.14
Smear grade	1.084 (0.489-2.403)	0.84

Variables with p-value less than 0.3 were included in the multivariate model; TTP= time to culture positivity.

Table 5.4: Adverse events reported by patients who received bedaquiline-based regimen and those who received bedaquiline-delamanid combination regimen. Data is n (%).

Adverse event	Patients who received bedaquiline-based (n=82)	Patients who received bedaquiline and delamanid (n=40)	p-values
Dizziness/disorientation	12 (14.6)	9 (22.5)	0.28
Psychosis	3 (3.7)	6 (15)	0.02*
Blurred vision	5 (6.1)	3 (7.5)	0.77
Hearing loss	41 (50.0)	18 (45.0)	0.60
Hypothyroidism	6 (7.3)	3 (7.5)	0.97
Peripheral neuropathy	18 (22.0)	12 (30.0)	0.33
Anaemia	28 (34.1)	15 (37.5)	0.72
Diarrhoea	7 (8.5)	6 (15)	0.28
Abdominal pain	16 (19.5)	5 (12.5)	0.34
Vomiting	20 (24.4)	8 (20.0)	0.59
Nausea	16 (19.5)	5 (12.5)	0.34
Elevated liver enzyme	23 (28.0)	13 (32.5)	0.61
Deranged renal function	17 (20.7)	9 (22.5)	0.82
Arthralgia	15 (18.3)	5 (12.5)	0.42

*33.3% of patients who had psychosis in the bedaquiline-delamanid group received terizidone and high dose isoniazid in their regimen compared to 11% in the bedaquiline group; both drugs are associated with increased risk of developing psychosis.

Table 5.5: QTcF profiles of patients who received bedaquiline-based regimen and those who received bedaquiline-delamanid combination regimen in the whole cohort and in HIV-infected patients. Data is n (%) and median (interquartile range).

Variable	Patients who received bedaquiline-based regimen	Patients who received bedaquiline-delamanid combination therapy	p-values
Whole cohort	**n=82**	**n=40**	
Baseline QTcF	408 (IQR 388-425)	419 (IQR 389-436)	0.32
Maximum QTcF change from baseline	27 (IQR 13-42)	23 (IQR 8-54)	0.11
Patients with QTcF increment from baseline greater than 60 ms	6 (7.3)	7 (20.6)	<0.001
Patients with at least one QTcF greater than 450 ms	16 (19.5)	15 (44.1)	<0.001
Patients with at least one QTcF greater than 500 ms	0 (0)	0 (0)	N/A*
HIV-infected patients	**n=42**	**n=22**	**p-values**
Baseline QTcF	407 (IQR 385-428)	417 (IQR 378- 436)	0.54
QTcF change from baseline	32 (IQR 14-44)	22 (IQR 6-56)	0.19
Patients with QTcF difference greater than 60 ms	5 (11.9)	4 (18.2)	0.49
Patients with QTcF greater than 450 ms	11 (26.2)	7 (31.8)	0.32
Patients with QTcF greater than 500 ms	0 (0)	0 (0)	N/A*
HIV uninfected patients	**n=40**	**n=18**	
Baseline QTcF	409 (IQR 394- 419)	419 (IQR 393-429)	0.21
QTcF change from baseline	22 (IQR 12- 37)	32 (IQR 13- 52)	0.48
Patients with QTcF difference greater than 60 ms	1	3	0.06
Patients with QTcF greater than 450 ms	5	8	0.02
Patients with QTcF greater than 500 ms	0	0	N/A*

N/A*: Not applicable

Table 5.6: Cox proportional hazard model for having at least one QTc value of greater than 450ms

Variables	Hazard ratio (95% C.I)	p-value
Univariate analysis		
Age (years)	1.031 (0.998- 1.066)	0.07
Gender (male)	1.221 (0.570- 2.614)	0.61
Weight (kg)	0.980 (0.948- 1.014)	0.24
Age at admission < 50 years	0.427 (0.146-1.248)	0.12
HIV-infection	1.043 (0.504- 2.158)	0.91
Previous TB treatment	1.195 (0.579- 2.466)	0.63
Previous treatment failures	1.304 (0.536- 3.173)	0.56
Days of admission	1.002 (1.000- 1.004)	0.06
Clofazimine treatment	0.731 (0.220- 2.426)	0.61
Delamanid treatment	3.668 (1.712- 7.859)	**0.001**
Moxifloxacin treatment	1.306 (0.619- 2.757)	0.48
Levofloxacin treatment	1.564 (0.748- 3.271)	0.24
Linezolid treatment	0.605 (0.244-1.501)	0.28
Number of medications	1.104 (0.884- 1.380)	0.38
Number of adverse events	0.976 (0.832- 1.145)	0.77
5 likely effective drugs	2.025 (0.761- 5.386)	0.16
TTP* < 7 days	0.777 (0.184- 3.282)	0.73
SMG$^{\#}$ > 2 plusses	1.504 (0.609- 3.714)	0.38
Multivariate analysis		
5 likely effective drugs	3.167 (0.995- 10.08)	0.05
Age	1.039 (1.000- 1.078)	**0.04**
Delamanid treatment	3.504 (1.544- 7.954)	**0.003**
Linezolid treatment	0.539 (0.200- 1.454)	0.22
Levofloxacin treatment	1.357 (0.494- 3.730)	0.55
Days of admission	1.001 (0.998- 1.004)	0.46
Weight	0.975 (0.937- 1.015)	0.23

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PART III: Summary and Conclusions

Chapter 6: Summary and conclusions

6.1. Introduction

This chapter highlights the major findings of the studies included in this thesis. The implication of the findings on policy formulation regarding the treatment guidelines for drug resistant tuberculosis and overall addition to knowledge are also discussed. In conclusion, recommendations are made with respect to national and international treatment guidelines for drug-resistant tuberculosis.

6.2. Summary of research findings

The research highlights the substantial contribution of new and repurposed drugs towards favourable outcomes in patients with drug-resistant TB and also provide insights on their safety. Although these findings have been discussed in each of the empirical research papers in chapters three, four and five, this section will provide a summary of the major findings in each paper, discuss their implications and inter-relatedness, and make a unified conclusion.

In the first study, treatment outcomes and adverse events profile were compared between programmatically treated XDR-TB patients who received the novel drug, bedaquiline, as part of their treatment regimen to patients treated with a non-bedaquiline containing regimen. Bedaquiline inclusion in a treatment regimen for DR-TB was associated with a five-fold improvement in treatment outcomes. Although some patients reported increased QTc prolongation which is one of the key adverse events related to bedaquiline use [1], none of them reached the threshold value of 500 ms (a threshold that strongly predicts short and long term mortality) or developed symptoms that necessitated bedaquiline withdrawal. Bedaquiline was also well tolerated in HIV-infected patients and it significantly improved their survival rates.

Linezolid, a repurposed drug, is a key drug in WHO's currently proposed all-oral regimen for MDR-TB [2]. However, it is strongly associated with severe adverse events leading to linezolid dosage reduction or permanent withdrawal from the treatment regimen. The second study evaluated the frequency of these adverse events and assessed the impact on patient-related treatment outcomes. The study showed that over one-third of patients had interrupted linezolid following the development of adverse events which occurred at predictable time frames. The study also showed that HIV co-infection and high mycobacterial load (TTP) at the time of diagnosis were strong predictors of linezolid interruption. However, linezolid interruption did not appear to affect treatment outcomes of affected patients, probably because they had a median 232 days exposure to linezolid which protected the regimen enough to ensure favourable outcome

In view of their synergistic cardiotoxicity, bedaquiline and delamanid were not routinely recommended for simultaneous use in the current guideline. However, in situations where an effective regimen cannot otherwise be constituted, the use of regimens containing bedaquiline and linezolid becomes inevitable, and more so in patients with FQ-resistant TB. Patients in whom a combination of bedaquiline and delamanid may be necessitated include those who have extensive resistance and recurrent poor treatment outcomes with limited treatment options. Thus, the third study examined the efficacy and safety of concurrent use of bedaquiline and delamanid in this group of drug-resistant TB patients. Despite having poor prognostic factors, patients who received the combination therapy had comparable treatment outcomes and adverse events profiles with less-sick patients who received a bedaquiline-based regimen. Even though patients receiving concurrent treatment with bedaquiline and delamanid had significantly greater proportion of prolonged QTc duration, none of them had drug withdrawal or developed cardiotoxic symptoms. Findings were essentially similar in HIV co-infected patients on ARV treatment.

6.3. Implications of research findings

South Africa is a country with one of the highest incidences of TB in the world. For several years running, TB is ranked as one of the leading causes of all mortality in the country, mostly aggravated by the increasing prevalence of drug-resistant TB. Although government has continued to improve on the treatment guidelines, all the efforts are being drowned by the persistence and high rates of drug-resistant TB in almost all the provinces in the country. In 2012 the South Africa's Medicines Control Council (MCC) now called South Africa Health Products Regulatory Authority (SAHPRA) approved Clinical Access to Bedaquiline Program (CAP) for XDR-TB patients and implementation started in 2013 [2]. Treatment centres for M/ XDR-TB patients were set-up in the country's nine provinces to run this program for patients who qualified for the treatment. This provided a new hope for patients and also improved the capacity for research into the efficacy and safety of bedaquiline, including those reported in this thesis.

A few years after the launch of the Clinical Access to Bedaquiline Program, two major publications, the first being study 1 reported in this thesis, and another one published three months later, reported treatment outcomes of drug resistant TB patients who received bedaquiline-based regimen in South Africa [3, 4]. Both studies highlighted the significant improvement in clinical outcomes of patients who received bedaquiline as compared to those who did not. Consequently, the National Department of Health requested that the data be presented at its strategic meeting and this led to a major shift in the treatment guidelines for the treatment of drug-resistant TB in the country. Following the promising preliminary results of a bedaquiline-based regimen reported from this and other researches, the South African National TB program (SA-NTP) with support from the WHO, successfully petitioned the manufacturer (Janssen / Johnson and Johnson) to reduce pricing of bedaquiline. This resulted in bedaquiline being introduced into SA National TB Program; it is recommended as a

frontline drug in a regimen for all new patients with M/XDR-TB, MDR-TB patients with HIV co-infection, including those on ARV, however, with a change of efavirenz to nevirapine to prevent drug-drug interactions that could reduce bedaquiline efficacy. The roll-out of bedaquiline across the country was achieved by the end of 2018 [5]. Lastly, the positive South African experience of the use bedaquiline in DR-TB served as an exemplar for other low- and middle-income countries to procure bedaquiline at a discounted rate through the Global Drug Facility, which is now managed by the Stop TB Partnership [6, 7].

The incorporation of bedaquiline as part of a standardized MDR-TB regimen came hand-in-hand with measures to "protect" the drug against the development of resistance by the mycobacterium tuberculosis. This is achieved mainly in two ways (i) Ensuring patients who receive a bedaquiline-based regimen do not have resistance beyond MDR-TB (i.e. resistance to fluoroquinolones). This is achieved through the Hain MTBDRplus and sl assays using the clinical sample, with a follow up phenotypic DST being performed on the culture isolate, and (ii) Ensuring that bedaquiline is accompanied by at least 3-4 other likely effective drugs to prevent resistance amplification. This is achieved by the incorporation of linezolid for newly diagnosed M/XDR-TB. In fact, the roll-out in different provinces was dependent upon the availability of linezolid to complement the bedaquiline-based regimen [8]. However, several other factors may continue to drive the emergence of bedaquiline resistance including PK mismatch, poor penetration into certain TB lesions, compartments and cavities, population level PK variability, adherence, and health-system related issues.

Linezolid is effective but highly toxic when used for the treatment of drug-resistant TB with ~30% of patients interrupting treatment. Thus, it was unclear if incorporation into the standardized regimen would impact treatment outcomes. Study 2 reported in this thesis was therefore designed to inform on the tolerability of linezolid when used in conjunction with bedaquiline-based regimen in a programmatic setting. This study confirmed the time specific

toxicity that necessitated the withdrawal linezolid in a significant proportion of patient. Importantly, it also showed that despite the interruption, achieving favourable outcomes was possible, and that TB clinicians were capable of monitoring patients receiving linezolid by reacting appropriately to adverse events. Thus, this study provided important data to the South African National TB program and endorsed the use of linezolid in M/XDR-TB regimen. However, the optimal dose of linezolid (frequency and total drug) in individual patients remains unclear and role of therapeutic drug monitoring remains unexplored.

The most recent WHO recommendation for MDR-TB treatment placed delamanid in the third (least priority) group, only to be included in the regimen when drugs in groups A and B could not be used [9]. Although delamanid is currently classified as a group C drug according to the latest WHO classification, it becomes an important treatment option in patients where a regimen containing 4-5 effective drugs cannot be constituted. However, the looming concern with this drug is the synergistic cardiotoxicity when combined with bedaquiline. At the time there were scanty data supporting the concurrent use of delamanid and bedaquiline. Thus, study 3 was designed to answer this critical gap in the literature. This study confirmed the safety and efficacy of the bedaquiline-delamanid combination therapy, even in the patients with poor prognostic features and patients infected with HIV on HAART. Importantly, this study endorsed the decision by the South African National TB program to use delamanid in carefully selected M/XDR-TB patients (in whom an effective regimen cannot be constituted).

6.4. Other research directions

The current WHO treatment guideline for MDR-TB provided two treatment options for MDR-TB namely the short injection-based regimen and the long all-oral bedaquiline-based treatment regimen [8, 9]. It was not expressly stated which one was the preferred, leaving the choice to individual preference (after discussion with the TB clinician) and programmatic factors such

as availability of bedaquiline. The all-oral long treatment regimen comprises mainly the new and repurposed drugs (bedaquiline, linezolid, levofloxacin/ moxifloxacin, cycloserine/ clofazimine), recommended for 18-20 months; the short regimen which still contains an injectable agent (amikacin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol) is recommended for nine to 12 months. Following the unimpressive performance of the shorter regimen in the STREAM-1 trial ((likely due to the Hawthorne effect), South Africa announced a modified short regimen in June 2018, mainly to reduce toxicity associated with SLID and to leverage on the impressive outcomes associated with the bedaquiline-based regimen [8]. While this regimen will clearly reduce toxicity, associated with the injectables, its efficacy remains to be tested in programmatic settings. This is currently being evaluated through our group.

Furthermore, bedaquiline is currently being evaluated with different drug combinations and treatment durations in ongoing clinical trials. STREAM-2 clinical trial, a phase 2b trial is comparing a six and nine months bedaquiline containing regimen against the WHO regimen. The NExT RCT trial is a phase 3 trial is evaluating the efficacy of a six to nine months injection-free bedaquiline-based regimen compared to standard of care. In the NiX-TB trial, a three-drug regimen including bedaquiline, pretomanid and a high-dose linezolid was used to treat XDR-TB and MDR-TB patients for six months, with an option to extend treatment to 9 months. Preliminary analysis of data from this single-arm study shows good outcomes with this regimen, suggesting a potent synergistic effect of bedaquiline when used in combination with pretomanid and high-dose linezolid with favourable treatment outcome rates of ~90% (compared to ~70% using a bedaquiline-linezolid based regimen in programmatic settings shown in study 1 of this thesis). Several other clinical trial like DELIBERATE and TB-PRATECAL, to mention a few, are also currently ongoing, all investigating the efficacy of

different combination options for bedaquiline. It is expected that these trials will further define the optimal use of bedaquiline in M/XDR-TB.

Given the predictability of time frame, by when adverse events would occur following the initiation of linezolid in a bedaquiline-based regimen, a shorter duration of linezolid inclusion in the regimen is currently being advocated in South Africa, primarily to allow for confirmation phenotypic DST to flouroquinolone, however, there is some evidence to suggest that linezolid may be most useful earlier in the regimen prior to the onset of neuropathic toxicity. This question needs further exploration.

6.5. Conclusions

This research investigated the efficacy and safety of new (bedaquiline and delamanid) and repurposed (linezolid) drugs for the treatment of M/XDR-TB. These drugs have led to substantial improvement in the favourable outcome rate of patients who had previously been deemed as therapeutically destitute. The research outlined in this thesis has provided the regulatory authorities with ammunition to effect policy change and positively impact the lives of hundreds of patients suffering from the scourge of drug-resistant TB. However, despite the dramatic increase in the favourable outcome rate, one must not lose sight of the significant proportion (~30%) who failed treatment despite having access to the new and repurposed agents. These patients have very limited treatment options and are often discharged into the communities where they may continue to spread highly resistant disease. Research needs to focus on developing novel and repurposed agents coupled with innovative drug delivery strategies (inhaled antibiotics) to treat patients who are currently classified as "treatment destitute". Thus, despite achieving significant gains in the fight against drug-resistant TB, our efforts cannot slow down because the fight against TB is far from over. In addition to developing novel and repurposed agents for the treatment of drug-resistant TB, we need to

focus our energy and finances to enhance preventive measure against TB in general (e.g. vaccine development, focused prophylaxis and infection control measures) and develop better diagnostic tools(point-of-care) and treatment for drug-sensitive TB. Lastly considering that ~30% of TB cases globally (~150000 cases in South Africa, including ~6000 cases of rifampicin resistant TB) remain undiagnosed or unreported, our strategies to fight TB must incorporate a component of active case finding. In conclusion, the fight against TB requires a multi-pronged approach incorporating factors highlighted above. There is a glimmer of hope that we are now beginning to turn the tide, however, the fight is far from over.

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Appendix A: Chapter three supplementary material

Table S3.1: Grading of adverse events severity[1]

Grade 0	No Adverse events
Grade 1	Mild adverse event, requiring no intervention
Grade 2	Moderate adverse event requiring either changing the dose or frequency of the offending drug, or prescribing another drug to manage the adverse event
Grade 3	Severe adverse event, enough to stop the offending drug
Grade 4	Life threatening or disabling adverse event
Grade 5	Death resulting from the adverse event

[1]Grading was done according to the modified American National Institute of Health common terminology of criteria for adverse events

Table S3.2: Treatment-related outcome definitions applied, as adapted from the 2013 WHO revised definitions and reporting framework for TB guidelines, and the core research definitions for drug-resistant TB clinical trials recommended by Furin *et al* [172, 173].

Treatment outcome		**Definition**
Favourable outcome	Cured	Treatment completed, as recommended by the National TB programme, without evidence of failure or an unfavourable outcome as defined below. Three or more consecutive negative sputum cultures, taken at least 30 days apart, after the intensive phase (up to 12 months from the initiation of treatment), or a participant's last two culture results at the end of treatment are negative.
	Completed treatment	Treatment completed, as recommended by the National TB programme, without evidence of failure or an unfavourable outcome, however no record of three or more consecutive negative sputum cultures, taken at least 30 days apart, after the intensive phase (up to 12 months from the initiation of treatment), or a participant's last two culture results at the end of treatment are not recorded as negative.
Unfavourable outcome	Treatment failure	Treatment terminated (stopping of two or more drugs), or the need for permanent regimen change of at least two anti-TB drugs (stoppage of or the change one drug in the case of linezolid or bedaquiline) because of one or more of the following: i) lack of sputum culture conversion, or culture reversion after initial conversion, or culture positivity after month 6 [173], (ii) drug-related adverse events (AEs), (iii) evidence of additional acquired drug resistance precluding the composition of a regimen of at least 4 likely effective drugs. (In the case culture positivity during or after month 6, only 1 positive culture is deemed to be sufficient when considered in the context of other biomarkers including weight, radiological disease extent, symptoms etc, based on the core research definitions for drug-resistant TB clinical trials recommended by Furin *et al* [173].)
	Died while on treatment infection)	A patient who died for any reason while on any TB treatment, or within 7 days of termination of treatment. For post treatment time-specific outcome all-cause mortality will be used. Death superseded any treatment outcome at a specific time point.
	Recurrence (relapse or re-	Two or more consecutive positive sputum cultures, at least 7 to 30 days apart, subsequent to the outcome of 'Cure' or 'Treatment Complete'. Genotyping is required to distinguish relapse from re-infection.
	Defaulted	A patient who interrupted treatment for 2, or more, consecutive months and who did not restart treatment but remained hospitalised or traceable in the community.
Indeterminate	Loss to follow up	A patient who interrupted treatment for 2, or more, consecutive months and who did not restart treatment but remains untraceable despite intensive and best efforts to find or track down the patient.
	Ongoing treatment	A patient for whom no treatment outcome can be assigned due to ongoing treatment in accordance with the National TB programme.

Table S3.3: Univariate Cox proportional hazard model for risk of death for all the XDR-TB patients (n=272).

Variable	Hazard ratio (95% C.I.)	p-value
Age at XDR-TB diagnosis (years)	1.00 (0.99- 1.02)	0.51
Weight <50	1.68 (1.22- 2.32)	0.002
Duration of TB treatment (days)	0.98 (0.98- 0.98)	<0.001
Gender (male)	0.93 (0.67- 1.29)	0.66
Median number of days of admission	1.00 (1.00- 1.00)	0.03
Median number of anti-TB drugs received	0.92 (0.83- 1.03)	0.14
*HIV Infected	1.17 (0.85- 1.61)	0.35
Previous TB treatment	1.60 (1.04- 2.44)	0.03
Amikacin	2.37 (0.58- 9.59)	0.23
Capreomycin	3.51 (2.09- 5.91)	<0.001
Kanamycin	1.80 (1.30- 2.50)	<0.001
[a]Any aminoglycosides	4.96 (2.60- 9.44)	<0.001
PAS	0.35 (0.19- 0.68)	0.002
Moxifloxacin	0.91 (0.66- 1.26)	0.57
Levofloxacin	0.17 (0.09- 0.33)	<0.001
[b]Third generation quinolones	0.46 (0.33- 0.64)	<0.001
Clofazimine	0.36 (0.25- 0.51)	<0.001
Linezolid	0.15 (0.07- 0.34)	<0.001
Bedaquiline	0.17 (0.09- 0.32)	<0.001
Ethionamide	4.33 (2.34- 8.03)	<0.001
Amoxycillin	0.94 (0.46- 1.92)	0.86
Age at XDR-TB diagnosis (years)	1.00 (0.99- 1.02)	0.51

*One patient refused HIV testing, n=271; [a]amikacin, capreomycin and/or kanamycin; [b]moxifloxacin or levofloxacin.

Table S3.4: Univariate Cox proportional hazard model for risk of death for HIV-infected patients in both groups (n=134).

Variable	Hazard ratio (95% C.I.)	p-value
Age at XDR-TB diagnosis (Years)	1.00 (0.97- 1.03)	0.87
Gender (Male)	0.89 (0.56- 1.41)	0.61
Weight <50kg at admission	1.65 (1.04- 2.62)	0.03
Previous TB treatment	1.44 (0.77- 2.68)	0.25
On ARV treatment	0.65 (0.28- 1.50)	0.31
*Median CD4 count <200 cells/µl at admission	1.14 (0.72- 1.81)	0.58
Median number of anti-TB drugs received	0.94 (0.79- 1.11)	0.47
Median number of days of admission	1.00 (1.00- 1.00)	0.01
Median duration of TB treatment (in days)	0.98 (0.98- 0.98)	<0.001
Bedaquiline	0.20 (0.08- 0.45)	<0.001
Clofazimine	0.31 (0.19- 0.50)	<0.001
Linezolid	0.19 (0.08- 0.47)	<0.001
Capreomycin	3.42 (1.70- 6.89)	<0.001
Kanamycin	2.32 (1.46- 3.68)	<0.001
Amikacin	1.64 (0.23- 11.85)	0.62
[a]Any aminoglycosides	4.10 (1.87- 8.97)	<0.001
Levofloxacin	0.21 (0.09- 0.48)	<0.001
Moxifloxacin	1.02 (0.64- 1.62)	0.93
[b]3rd Generation fluoroquinolones	0.45 (0.28- 0.73)	<0.001
PAS	0.34 (0.14- 0.85)	0.02
Ethionamide	4.34 (1.87- 10.04)	<0.001
Amoxycillin	1.43 (0.45- 4.56)	0.54

*2 patients did not have CD4 count done at admission (n=132); [a]amikacin, capreomycin and/or kanamycin; [b]moxifloxacin or levofloxacin.

Table S3.5: Multivariate Cox proportional hazard model for risk of death in both groups excluding colinear variables; A) all the XDR-TB patients (n=271), B) HIV-infected patients (n=132). Univariate analyses are shown in supplementary Tables S3 and S4 for the whole cohort and the HIV-infected subgroups respectively.

Variables	**Hazard ratio (95% C.I.)**	**p-value**
I) All the XDR-TB patients (n=271)		
Weight <50kg at admission	1.96 (1.38- 2.77)	<0.001
Gender (male)	1.06 (0.76- 1.49)	0.72
[A]HIV-infected	1.49 (1.05- 2.11)	0.03
Previous TB treatment	1.08 (0.69- 1.67)	0.74
Number of anti-TB drugs received	0.83 (0.72- 0.96)	0.01
[B]Bedaquiline	0.14 (0.06- 0.30)	<0.001
Clofazamine	0.80 (0.47- 1.37)	0.42
[C]Third generation fluoroquinolones	1.10 (0.68- 1.76)	0.70
II) HIV-infected patients (n=132)		
Weight <50kg at admission	1.86 (1.13- 3.08)	0.02
Gender (male)	0.72 (0.43- 1.20)	0.21
Number of anti-TB drugs received	0.86 (0.66- 1.12)	0.26
[D]Any aminoglycoside	0.05 (0.00- 0.58)	0.02
On ARV treatment	1.29 (0.49- 3.38)	0.6
[E]CD4 count <200 cell/µl	1.53 (0.92- 2.54)	0.11
[B]Bedaquiline	0.01 (0.00- 0.16)	<0.001
Clofazamine	0.63 (0.3- 1.33)	0.23
Kanamycin	1.50 (0.88- 2.55)	0.14
Previous TB treatment	1.21 (0.61- 2.38)	0.58

A) One patient refused testing; B) 53 of the 68 (77.9%) patients who received bedaquiline also received linezolid; C) 3rd generation fluoroquinolones = moxifloxacin and levofloxacin; D) Any aminoglycoside = amikacin, capreomycin and kanamycin. E) 2 patients did not have CD4 count done at admission (n=132).

Table S3.6: Univariate Cox proportional hazard model for risk of unfavourable treatment outcome for all the XDR-TB patients (n=270).

Variable	**Hazard ratio (95% C.I.)**	**p-value**
Age at XDR-TB diagnosis (years)	1.00 (0.99- 1.02)	0.51
Weight <50	1.47 (1.11- 1.95)	0.01
Duration of TB treatment (days)	0.98 (0.98- 0.98)	<0.001
Gender (male)	1.05 (0.79- 1.39)	0.74
Median number of days of admission	1.00 (1.00- 1.00)	0.02
Median number of anti-TB drugs received	0.94 (0.86- 1.03)	0.21
*HIV Infected	1.07 (0.80- 1.41)	0.66
Previous TB treatment	1.41 (0.99- 2.00)	0.06
Amikacin	2.06 (0.51- 8.33)	0.31
Capreomycin	2.57 (1.70- 3.86)	<0.001
Kanamycin	1.59 (1.20- 2.11)	<0.001
[a]Any aminoglycoside	3.15 (1.97- 5.02)	<0.001
PAS	0.40 (0.22- 0.73)	0.003
Moxifloxacin	0.96 (0.72- 1.27)	0.76
Levofloxacin	0.32 (0.20- 0.49)	<0.001
[b]Third generation quinolones	0.58 (0.44- 0.77)	<0.001
Clofazimine	0.49 (0.37- 0.66)	<0.001
Linezolid	0.30 (0.18- 0.51)	<0.001
Bedaquiline	0.31 (0.20- 0.48)	<0.001
Ethionamide	2.96 (1.87- 4.66)	<0.001
Amoxycillin	1.15 (0.66- 2.03)	0.62

*one patient refused HIV testing, n=269; [a]amikacin, capreomycin and/or kanamycin; [b]moxifloxacin or levofloxacin.

Table S3.7: Univariate Cox proportional hazard model for risk of unfavourable treatment outcome in HIV-infected patients from both groups (n=133).

Variable	**Hazard ratio (95% C.I.)**	**p-value**
Age at XDR-TB diagnosis (Years)	1.00 (0.97- 1.02)	0.79
Gender (Male)	1.00 (0.66- 1.51)	0.99
Weight <50kg at admission	1.75 (1.15- 2.66)	0.008
Previous TB treatment	1.34 (0.78- 2.30)	0.30
On ARV treatment	0.94 (0.41- 2.20)	0.89
*Median CD4 count <200 cells/µl at admission	1.10 (0.72- 1.67)	0.66
Median number of anti-TB drugs received	0.91 (0.78- 1.07)	0.24
Median number of days of admission	1.00 (1.00- 1.00)	<0.001
Median duration of TB treatment (in days)	0.98 (0.97- 0.98)	<0.001
Bedaquiline	0.30 (0.16- 0.57)	<0.001
Clofazimine	0.38 (0.25- 0.59)	<0.001
Linezolid	0.28 (0.14- 0.57)	<0.001
Capreomycin	2.42 (1.36- 4.31)	<0.001
Kanamycin	1.94 (1.27- 2.96)	<0.001
Amikacin	1.47 (0.20- 10.63)	0.70
[a]Any aminoglycoside	2.85 (1.54- 5.26)	<0.001
Levofloxacin	0.32 (0.17- 0.60)	<0.001
Moxifloxacin	1.02 (0.67- 1.54)	0.93
[b]3rd Generation fluoroquinolones	0.54 (0.35,0.83)	0.004
PAS	0.35 (0.15,0.80)	0.01
Ethionamide	3.18 (1.64,6.17)	<0.001
Amoxycillin	1.68 (0.61,4.61)	0.31

*2 patients did not have CD4 count done at admission (n=131); [a]amikacin, capreomycin and/or kanamycin; [b]moxifloxacin or levofloxacin.

Table S3.8: Multivariate Cox proportional hazard model for risk of unfavourable treatment outcome in both groups A) all the XDR-TB patients (n=271), B) HIV-infected patients in the (n=132).

Variables	**Hazard ratio (95% C.I.)**	**p-value**
I) All the XDR-TB patients (n=271)		
Weight <50kg at admission	1.72 (1.27- 2.33)	<0.001
Gender (male)	1.19 (0.88- 1.60)	0.26
[A]HIV-infected	1.25 (0.92- 1.70)	0.15
Previous TB treatment	1.05 (0.72- 1.52)	0.81
Number of anti-TB drugs received	0.85 (0.76- 0.96)	0.01
[B]Bedaquiline	0.24 (0.14- 0.42)	<0.001
Clofazamine	0.92 (0.58- 1.46)	0.74
[C]Third generation fluoroquinolones	1.13 (0.74- 1.73)	0.57
II) HIV-infected patients (n=132)		
Weight <50kg at admission	2.21 (1.39- 3.51)	<0.001
Gender (male)	0.83 (0.52- 1.33)	0.44
Number of anti-TB drugs received	0.8 (0.63- 1.01)	0.06
[D]Any aminoglycoside	0.04 (0.00- 0.45)	0.008
On ARV treatment	1.59 (0.61- 4.14)	0.34
[E]CD4 count <200 cell/µl	1.37 (0.87- 2.17)	0.17
[B]Bedaquiline	0.01 (0.00- 0.12)	<0.001
Clofazamine	0.84 (0.44- 1.63)	0.61
Kanamycin	1.24 (0.75- 2.05)	0.41
Previous TB treatment	1.26 (0.69- 2.31)	0.46

*one patient refused testing; **2 patients did not have CD4 count done at admission (n=132).

Table S3.9: Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of culture negativity at specific time points to predict A) survival and B) favourable treatment outcome for all patients.

Month	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Number of patients considered
A) Survival as dependant variable					
2	77.8	70.5	56	86.8	166
3	86.4	64	53.1	90.9	184
6	98	65.7	58.5	98.5	148
12	97.3	71.4	69.2	97.6	93
18	97.4	62.5	80.9	93.8	63
24	100	71.4	76.5	100	27
B) Favourable treatment outcome as dependant variable					
2	66.3	74.4	70.7	70.3	166
3	78.6	77.9	80.2	76.1	184
6	88.5	81.4	84.1	86.4	148
12	83.6	84.2	88.5	78	93
18	87.8	71.4	91.5	62.5	63
24	78.9	75	88.2	60	27

Table S3.10: Comparisons of treatment outcomes (A) and survival (B) between the Bdq and non-Bdq treatment groups with patients who died within the first two months following diagnosis excluded.The results show that our conclusions remain unchanged.

A) Comparison of Bdq and non-Bdq treatment groups by outcomes			
Variable	BDQ (n=62)	Non-BDQ (n=172)	p value
Favourable (cured/completed treatment)	45 (73%)	27 (15%)	
Unfavourable outcome (treatment failed, deceased)	17 (27%)	151 (85%)	<0.001
B) Comparison of Bdq and non-Bdq treatment groups by survival			
Variable	BDQ (n=62)	Non-BDQ (n=180)	p value
Alive	58 (94%)	65 (36%)	<0.001
Deceased	4 (6%)	115 (63%)	

Appendix B: Chapter four supplementary material

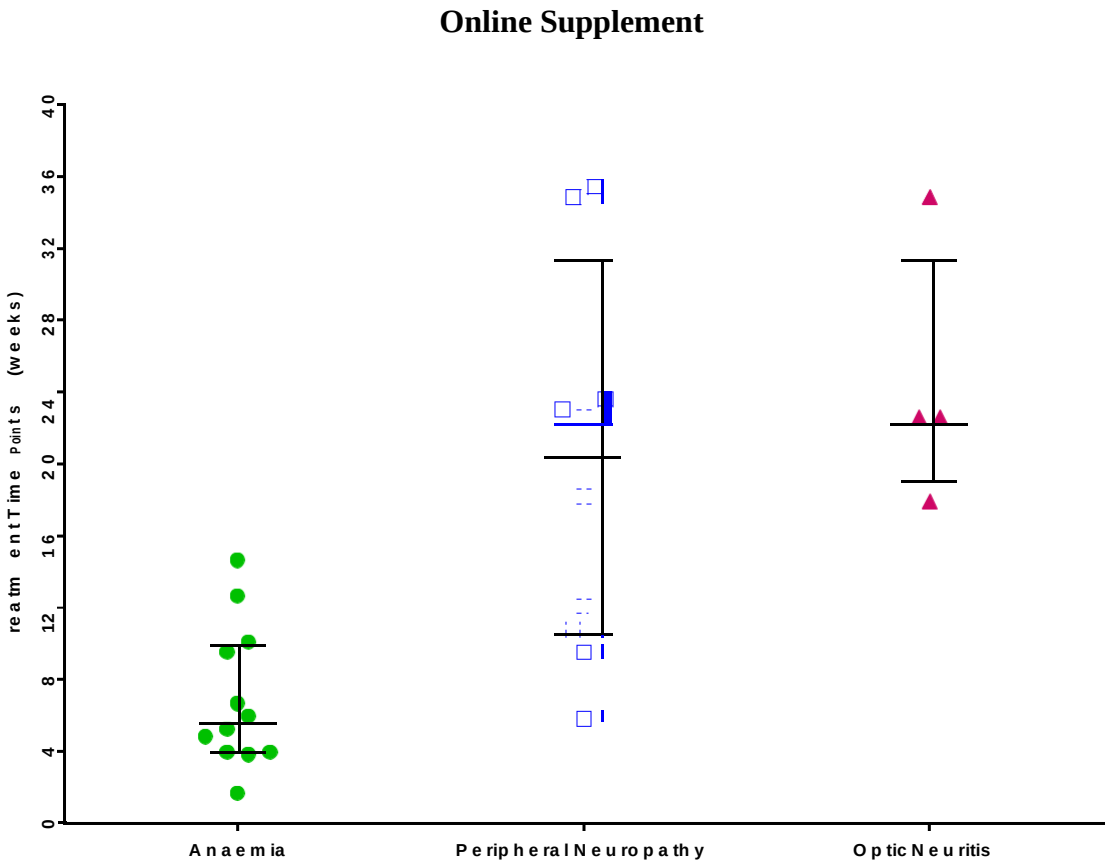
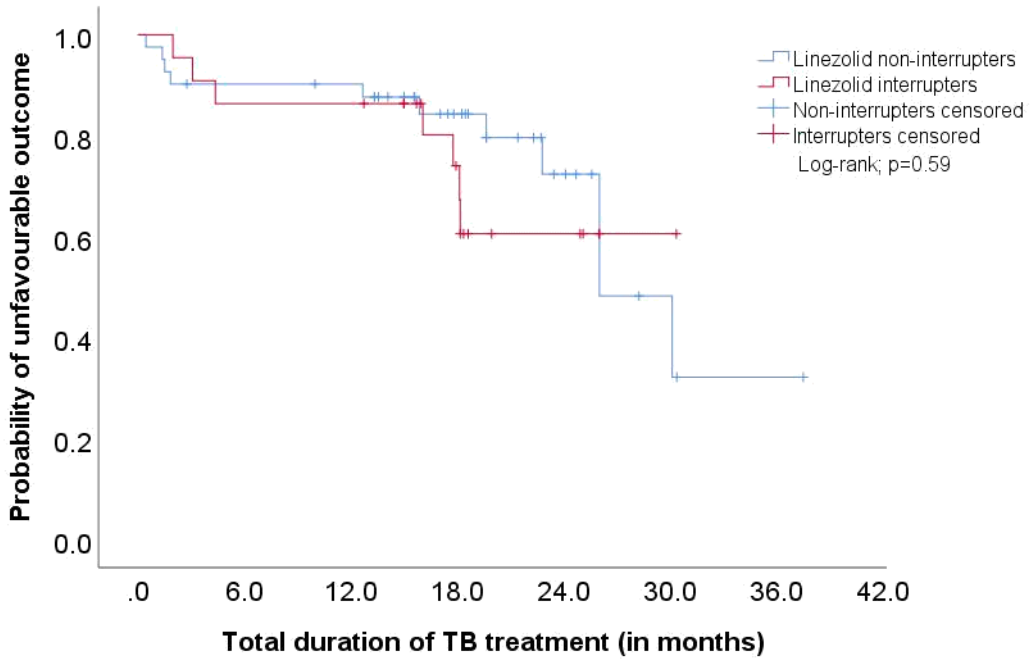


Figure S4.1: Graphical presentation of temporal relationship between linezolid initiation and development of adverse events (p=0.007).



Number at risk

Linezolid interrupters	22	19	19	11	5	1	0	0
Non-interrupters	41	37	37	22	9	3	1	1

Figure S4.2: Kaplan-Meier estimate for the probability of unfavourable outcome in linezolid interrupters and non-interrupters

Table S4.1: Univariate Cox proportional hazard model for the risk of unfavourable outcome and linezolid interruption in the whole cohort.

Unfavourable outcome (n=18)	**Hazard Ratio (95% C.I.)**	**p-value**
Linezolid interruption	0.769 (0.297- 1.994)	0.59
Linezolid treatment < 3 months	7.319 (2.799- 19.143)	<0.001
Linezolid treatment > 3 months	0.137(0.052- 0.357)	<0.001
Linezolid treatment 3-6 months	0.423 (0.056- 3.224)	0.41
Linezolid treatment > 6 months	0.248 (0.096- 0.644)	0.004
Linezolid treatment > 12 months	0.436 (0.143- 1.333)	0.15
Linezolid interruption (n=22)		
Levofloxacin	1.067 (0.140-8.128)	0.95
Moxifloxacin	1.597 (0.368-6.932)	0.53
PAS	1.134 (0.149-8.576)	0.90
Time from diagnosis to treatment initiation (days)	0.996 (0.988- 1.003)	0.28

Table S4.2: Univariate Cox proportional hazard model for the risk of death in the whole cohort.

Death (n=8)	**Hazard Ratio (95% C.I.)**	**p-value**
Weight(kg)	0.962 (0.894- 1.035)	0.30
Gender (male)	2.866 (0.684-12.004)	0.15
Days hospitalized	0.978 (0.963-0.993)	0.004
HIV-infected	5.019 (0.617- 40.797)	0.13
Age (years)	0.992 (0.923-1.066)	0.83
Previous tuberculosis treatment	0.968 (0.246- 3.892)	0.97
Levofloxacin treatment	0.047 (0.00-1445419.2)	0.73
Moxifloxacin treatment	26.4 (0.007-101373.6)	0.44
PAS treatment	0.046 (0.000-92955.8)	0.68
PZA treatment	0.047 (0.000-1445419.3)	0.73
Number of TB drugs	1.042 (0.621-1.750)	0.88
Smear grade (baseline)	1.907 (0.747- 4.867)	0.18
Time to positivity in days (baseline)	0.991 (0.898- 1.092)	0.85
Linezolid interruption	1.795 (0.449- 7.180)	0.41
Time from diagnosis to treatment initiation (days)	0.993 (0.977- 1.009)	0.38
Duration on linezolid (days)	0.988 (0.981-0.996)	0.002
Linezolid treatment > 3 months	0.158 (0.005- 0.454)	0.001
Linezolid treatment > 6 months	0.059 (0.007- 0.478)	0.008
Linezolid treatment > 12 months	0.241 (0.030- 1.962)	0.184

Table S4.3: Univariate Cox proportional hazard model for the risk of severe adverse events in the whole cohort.

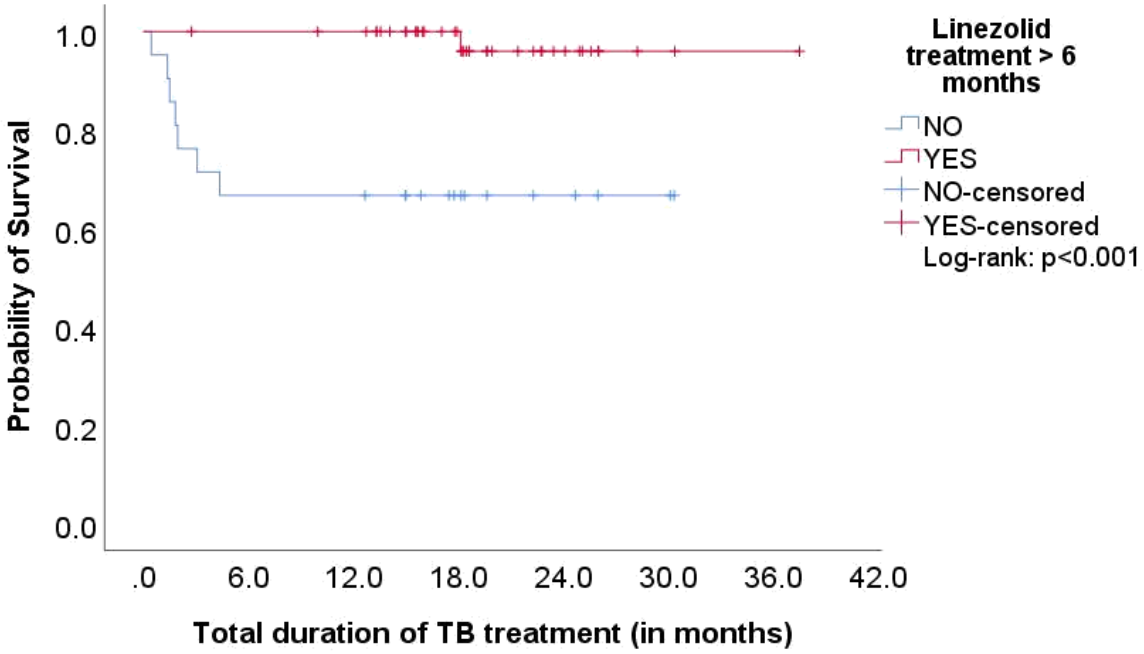
Severe adverse events (n=36)	**Hazard Ratio (95% C.I.)**	**p-value**
Weight (kg)	0.980 (0.949-1.011)	0.20
Gender (male)	1.708 (0.872-3.346)	0.12
Days hospitalized	0.996 (0.992-1.000)	0.06
HIV-infected	1.127 (0.575- 2.209)	0.73
Age (years)	1.020 (0.986-1.056)	0.25
Previous TB treatment	1.139 (0.819-1.583)	0.44
Duration on linezolid (days)	0.998 (0.996-1.000)	0.03
Smear grade (baseline)	1.215 (0.7999-1.846)	0.36
Time to positivity (baseline)	0.954 (0.908- 1.002)	0.06
Time from diagnosis to treatment initiation (days)	1.000 (0.999- 1.001)	0.79
Linezolid interruption	1.818 (0.941- 3.510)	0.08
Moxifloxacin	1.030 (0.397-2.675)	0.95
PAS	2.143 (0.647-7.099)	0.21
PZA	3.118 (0.729-13.329)	0.13
Number of TB drugs	1.717 (0.902-1.536)	0.23

Table S4.4: Univariate Cox proportional hazard model for achieving culture conversion in the whole cohort.

Culture conversion (n=55)	**Hazard Ratio (95% C.I.)**	**p-value**
Weight (kg)	0.996 (0.971-1.021)	0.74
Gender (male)	1.273 (0.722-2.243)	0.40
Days hospitalized	0.997(0.994-1.000)	0.06
HIV-infected	0.847 (0.489-1.467)	0.55
Age (years)	1.005 (0.978-1.034)	0.71
Previous TB treatment	1.182(0.691-2.022)	0.54
Duration on linezolid (days)	0.999 (0.997-1.000)	0.14
Levofloxacin	0.858 (0.207-3.563)	0.83
Moxifloxacin	0.736 (0.365-1.482)	0.39
PAS	0.720 (0.222- 2.333)	0.58
PZA	0.479 (0.114- 2.007)	0.31
Number of TB drugs	1.212 (0.978-1.502)	0.08
Linezolid interruption	0.855 (0.486-1.503)	0.59
Time to positivity in days (baseline)	0.927 (0.885- 0.971)	0.001
Time from diagnosis to treatment initiation (days)	1.000 (0.999- 1.001)	0.91
Smear grade (baseline)	1.178 (0.845- 1.641)	0.33
Completed linezolid treatment	1.193 (0.560- 2.542)	0.65

Table S4.5: Multivariate Cox proportional hazard model for culture conversion, severe adverse events and death in the whole cohort.

Culture conversion (n=55)	**Hazard Ratio (95% C.I.)**	**p-value**
Weight (kg)	1.018 (0.988- 1.049)	0.24
Gender (male)	1.098 (0.523- 2.306)	0.81
Number of TB drugs	1.256 (0.994- 1.586)	0.06
HIV-infected	0.653 (0.338- 1.262)	0.21
Duration on linezolid (days)	0.998 (0.996- 1.001)	0.24
PZA	8.360 (1.482- 47.166)	0.02
Time to positivity	0.923 (0.881- 0.966)	0.001
Days hospitalized	0.997 (0.994- 1.001)	0.15
Age	1.028 (0.996- 1.062)	0.09
Severe adverse events (n=36)		
Weight (kg)	1.001 (0.965- 1.040)	0.94
Gender (male)	1.046 (0.434- 2.524)	0.92
Number of TB drugs	1.275 (0.934- 1.741)	0.13
PZA	17.256 (2.534- 117.489)	0.004
Duration on linezolid (days)	0.997 (0.994- 1.000)	0.04
HIV-infected	2.003 (0.867- 4.625)	0.10
Days hospitalized	0.999 (0.994- 1.003)	0.51
Time to positivity in days (baseline)	0.944 (0.896-0.994)	0.03
Age (years)	1.048 (1.008-1.089)	0.02
Death (n=8)		
Weight (kg)	1.001 (0.923- 1.086)	0.98
Gender (male)	1.117 (0.177- 7.041)	0.49
Days hospitalized	0.989 (0.962- 1.018)	0.45
HIV-infected	2.874 (0.294- 28.074)	0.19
Linezolid treatment > 3 months	0.000 (0.000- 3480969)	0.94
Linezolid treatment > 6 months	0.582 (0.000- 7242987)	0.99
Linezolid treatment > 12 months	12227 (0.000- 1274987)	0.91



Number at risk

YES	42	42	42	25	10	2	1	1
NO	21	14	8	4	2	2	0	0

Figure S4.3: Kaplan-Meier survival estimate for patients who received linezolid for more than 6 months in their treatment regimen.

Appendix C: Chapter five supplementary material

Table S5.1: Culture conversion status of patients who received bedaquiline-based and those who received bedaquiline-delamanid combination regimens at different time points during treatment. Data is n (%).

	Patients who received bedaquiline-based regimen (n=82)	Patients who received delamanid-bedaquiline combination regimen (n=40)	p-values
Positive at baseline	52/82 (63.4)	26/40 (65.0)	0.86
Culture Conversion at 2 months	25/38 (65.8)	13/23 (56.5)	0.47
Culture Conversion at 6 months	33/36 (92.5)	18/22 (81.8)	0.26
Culture Conversion at 12 months	27/31 (87.1)	13/15 (86.7)	0.97

Patients who were culture negative at the point of recruitment were excluded from the analysis at 2, 6 and 12 months.

Table S5.2: Comparison of treatment outcomes between patients who received bedaquiline-based regimen and those who received delamanid-bedaquiline combination regimen. Data is (n)%

	Patients who received bedaquiline-based regimen (n=82)	Patients who received delamanid-bedaquiline combination regimen (n=40)	p-values
XDR-TB	n=67	n=19	
Favourable outcome	44 (65.7)	14 (73.7)	0.51
Unfavourable Outcome	23 (34.3)	5 (26.3)	
PRE-XDRTB	n=10	n=15	
Favourable outcome	4 (40)	9 (60)	0.32
Unfavourable Outcome	6 (60)	6 (40)	
MDR-TB	n=5	n=6	
Favourable outcome	4 (80)	4 (66.67)	0.62
Unfavourable Outcome	1 (20)	2(33.33)	

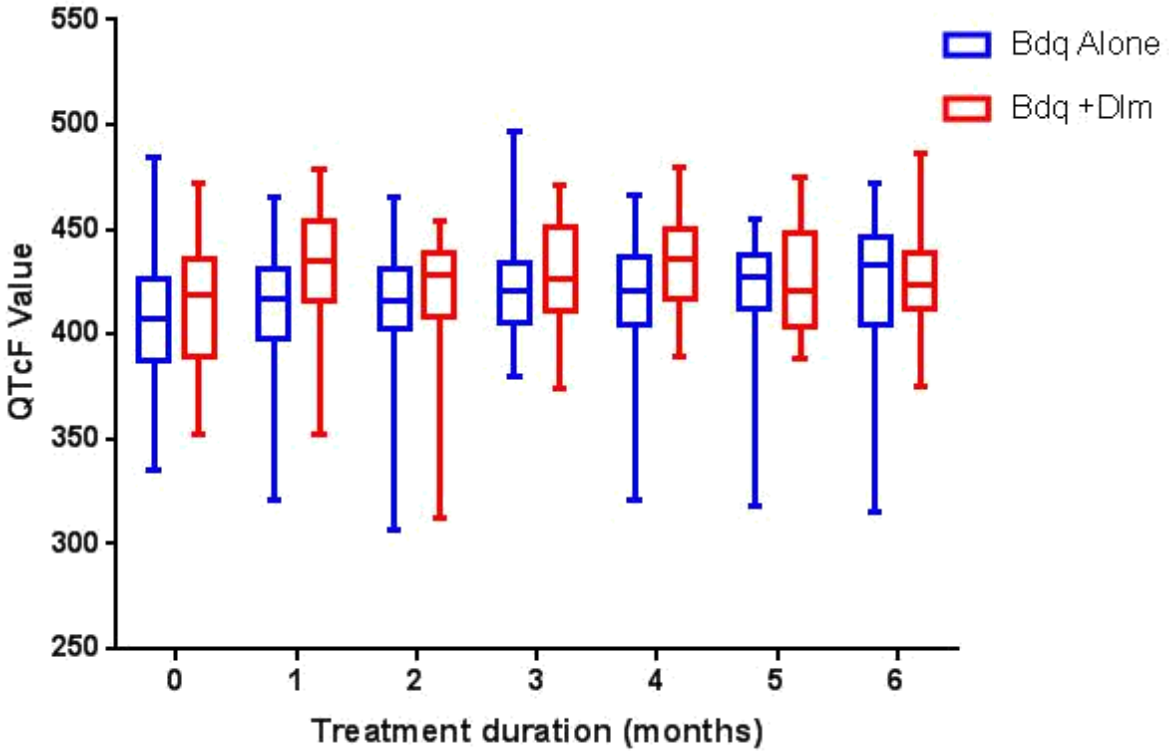


Figure S5.1: QTcF values at different time points during treatment with either bedaquiline-based regimen or delamanid-bedaquiline combination regimen. Boxes represent the median and IQR, while error bars represent range values

Table S5.3: (A) Univariate Cox proportional hazard model for developing unfavourable outcome in the HIV-infected patients.

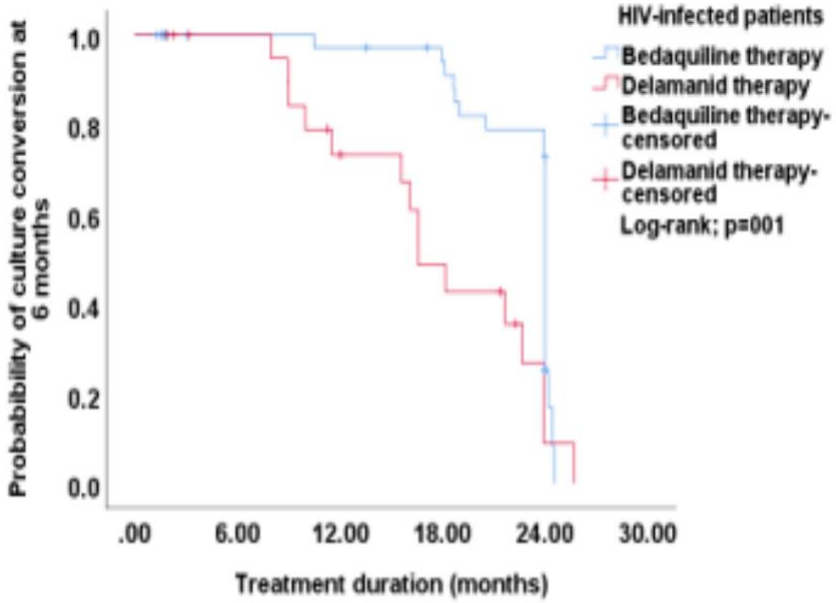
Variables	Hazard ratio (95% C.I)	p-value
Age (years)	1.013 (0.960- 1.068)	0.64
Gender (male)	1.173 (0.479- 2.871)	0.73
Weight (kg)	0,981 (0.945- 1.018)	0.31
Age at admission < 50 years	0.206 (0.058- 0.734)	0.02
Previous TB treatment	1.808 (0.748- 4.367)	0.19
Days of admission	0.993 (0.986- 0.999)	0.03
Clofazimine treatment	0.596 (0.080- 4.467)	0.62
Delamanid treatment	0.785 (0.485- 1.269)	0.32
Moxifloxacin treatment	1.262 (0.484- 3.293)	0.64
Levofloxacin treatment	0.883 (0.116- 6.717)	0.88
Any fluoroquinolone	0.047 (0.000-10560)	0.63
Linezolid treatment	0.416 (0.056- 3.109)	0.39
Delamanid-bedaquiline treatment	0.651 (0.248- 1.706)	0.38
Number of medications	1.099 (0.847- 1.426)	0.48
Number of adverse events	1.137 (0.960-1.347)	0.14
5 likely effective drugs	0.684 (0.395- 1.183)	0.17
Resistant to >5 drugs	2.688 (0.762- 9.482)	0.12
TTP* < 7 days	1.709 (0.570- 5.119)	0.34
SMG$^{\#}$ > 2 plusses	2.270 (0.752- 6.847)	0.15
(B) Multivariate Cox proportional hazard model for unfavourable outcome		
Age at admission < 50 years	0.333 (0.079-1.396)	0.13
Resistant to >5 drugs	4.725 (1.041-21.43)	0.04
Previous TB treatment	2.181 (0.810- 5.871)	0.12
Days of admission	0.990 (0.982- 0.998)	0.02
5 likely effective drugs	0.465 (0.142- 1.520)	0.21
Number of adverse events	1.173 (0.949- 1.449)	0.14
SMG$^{\#}$ > 2 plusses	2.442 (0.690- 8.640)	0.17

Table S5.4: Adverse events reported by HIV-infected patients who received bedaquiline-based regimen and those who received delamanid-bedaquiline combination regimen. Data is n (%).

Adverse event	Patients who received bedaquiline alone (n=42)	Patients who received bedaquiline and delamanid (n=22)	p-values
Dizziness/disorientation	5 (11.9)	4 (18.2)	0.49
Psychosis	2 (4.8)	4 (18.2)	0.08
Blurred vision	1 (2.4)	2 (9.1)	0.23
Hearing loss	20 (47.6)	8 (36.4)	0.39
Hypothyroidism	4 (9.5)	2 (9.1)	0.96
Peripheral neuropathy	6 (14.3)	7 (31.8)	0.098
Anaemia	7 (16.7)	11 (50)	0.005*
Diarrhoea	3 (7.1)	4 (18.2)	0.18
Abdominal pain	8 (19.0)	1 (4.5)	0.11
Vomiting	11 (26.2)	4 (18.2)	0.47
Nausea	9 (21.4)	3 (13.6)	0.45
Elevated liver enzyme	15 (35.7)	8 (36.4)	0.96
Deranged renal function	12 (28.6)	8 (36.4)	0.52
Arthralgia	8 (19)	3 (13.6)	0.59

*95.5% of patients in the bedaquiline-delamanid group received linezolid in their regimen compared to 88.1% in the bedaquiline group; linezolid is associated with increased risk of developing anaemia.

A



B

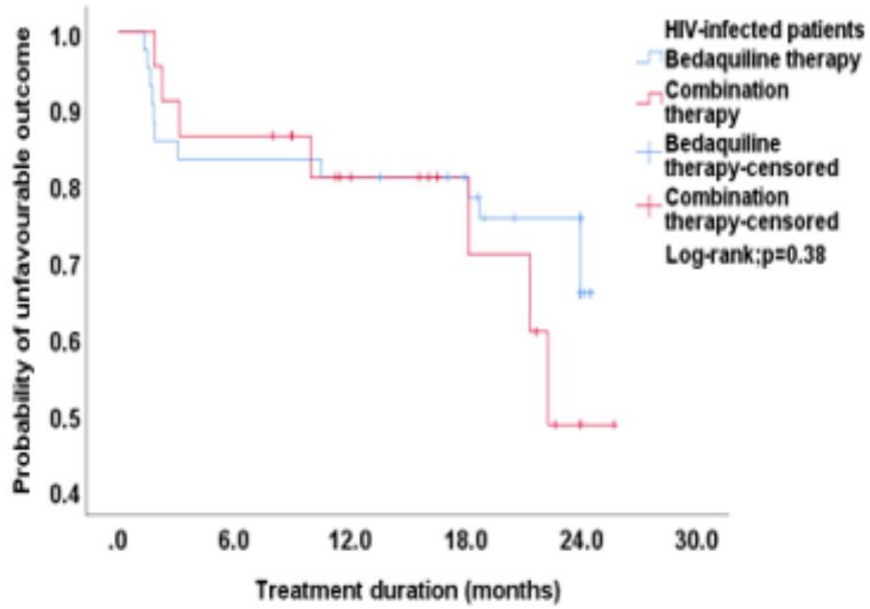


Figure S5.2 (A): Kaplan Meier estimate for the probability of culture conversion and (B) the probability of achieving an unfavourable outcome in HIV-infected patients who received bedaquiline-alone regimen and those who received delamanid-bedaquiline combination regimen.

Appendix D: Informed consent form

INFORMED CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

XDR and MDR TB: A study of treatment related outcomes, cost analysis and immune profiling

Principal Investigator: Prof K Dheda

Study number	Initials	Date of birth
……...	…….	……..

Thank you for your interest in this study. You have been identified as a possible participant because you are older than 18, you have been diagnosed with drug resistant Tuberculosis (TB) and you are receiving treatment from Brooklyn Chest Hospital.

For this study, we would like to gather certain information about you, your disease and management. This will help us to better understand how your type of TB works and will assist us to better manage TB in the future.

YOUR PARTICIPATION IN THE STUDY:

If you agree to take part in this study, the following will happen:

If you have started on TB treatment in the past two weeks, you will be asked to produce an additional sputum sample. This will be stored for future use in this research. We will use your file and laboratory results to gather information about you. We might also conduct an interview with you and ask you to complete a questionnaire.

The information we are collecting include the following:

-Your medical history (e.g. information about previous and current TB, treatment, side effects and other medical conditions)

-Laboratory and other investigation results

-Social information (e.g. smoking and substances, contacts, occupational history, forensic history, employment)

-Demographics (e.g. contact numbers, addresses, living arrangements

As part of the study, we will follow your condition and collect data about the outcome of yur disease. You will continue to receive the treatment that you are receiving now as prescribed by your doctor. If your treatment should stop working (treatment failure), we will also ask you to provide us with sputum sample so we can check why this has happened.

This will happen if your sputum sample taken after four months of treatment still shows TB. You will then be asked to produce an additional sputum sample at your 6 months, 9 months and one-year visits for study purposes. After that we will follow you up every six months to collect sputum, ask you questions about your health and treatment, and to check if your sputum results have changed. If available, we will then also compare this result with your first sputum sample.

This will help us to find out why your treatment is not working and if there are other medications that might help you.

Your initial sputum sample (if collected) will be stored for up to ten years and if you do not develop treatment failure, it will be discarded. Any future research using your sample, will first have to be approved by the human research ethics committee at the University of Cape Town.

There are also sub-studies linked to this study. If you are recruited by any of these studies, we will ask you to sign a separate consent form that will describe the tests and procedures done in those studies.

POSSIBLE RISKS OR BENEFITS

There will be no added risk if you choose to take part in this study. You might experience benefits from the study as we will be testing your sputum to see if there are medications that could treat your TB if your normal treatment should fail. It is also hoped that the information

we gather from this study will help us to better treat patients with a similar condition in the future.

CAN I SAY NO?

Yes, if you do not want to be a part of this study, you can withdraw at any time. This will not affect the management you receive.

REIMBURSEMENT

You will be compensated with R100 for each study related visit, to compensate you for time off work and any inconvenience caused by your participation in the study.

WILL MY MEDICAL INFORMATION BE KEPT CONFIDENTIAL?

We will do our best to protect the information we collect from you and your medical records. Information which identify you will be kept secure and restricted. If information from this research is published, or presented at scientific meetings, your name and other identifiers will not be used. Your privacy will be maintained throughout the study and nobody other than the doctors and nurses looking after you will know that you are participating in the study.

WHO CAN ANSWER MY QUESTIONS ABOUT THE STUDY?

You can talk to the study nurse or doctor about any questions, concerns, or complaints you have about this study that has not been answered. Contact the principal investigator, Professor K. Dheda, or a study doctor at the University of Cape Town, Lung Infection and Immunity Unit on 0214067654/6119 during office hours. You can also feel free to discuss this study with family or friends before signing the consent form.

If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about

the study, you may approach the chair of the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town:

Professor Mark Blockman, Faculty of Health Sciences, Research Ethics Committee, E52-23 Old Main Building, Groote Schuur Hospital, Observatory 7925. Tel 0214066492, 0214066411.

CONSENT STATEMENT

I have read the information about this study/ it has been read to me (circle which is appropriate) and I have had the opportunity to discuss the study and to ask questions. I freely choose to participate in the study.

I give permission for collection of my information and samples (tick the appropriate)

-Information in my hospital / clinic folder

-Interviews with me

-Completing a questionnaire

-Sputum samples

-Storage of sputum sample for up to 10 years

If you wish to be a part of this study, please sign below

Name of participant……

Participant's signature……. Date…….

Name of person obtaining consent……..

Signature……. Date…….

If the patient has been discharged before consent could be obtained, we shall obtain consent telephonically:

Telephonic consent from the patients (Number:……..)

Date…….

Time……

Consent given? Yes No

OR

If the patient cannot be reached or cannot give consent for any reason (death, too ill, etc.)

Telephonic consent from the patient's next of kin (Number…….)

Name……..

Relationship with the patient……

Date…….

Time…….

Consent given? Yes No

Co-signature of witness to telephonic consent

Name of witness……

Signature……

Date…….