Mycobactericidal effect of different regimens measured by molecular bacterial load assay among people treated for multidrug-resistant tuberculosis in Tanzania

Peter M. Mbelele¹,², Emmanuel A. Mpolya², Elingarami Sauli², Bariki Mtayfa³, Nyanda E. Ntinginya³, Kennedy K. Addo⁴, Katharina Kreppel², Sayoki Mfinanga⁵, Patrick P.J. Phillips⁶, Stephen H. Gillespie⁷, Scott K. Heysell⁸, Wilber Sabiiti⁷ and Stellah G. Mpagama¹,²

Affiliations

¹ Kibong’oto Infectious Diseases Hospital (KIDH), Siha, Kilimanjaro, Tanzania
² Department of Global Health and Biomedical Sciences, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania
³ National Institute for Medical Research, Mbeya Medical Research Centre, Tanzania
⁴ Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana
⁵ National Institute for Medical Research, Muhimbili Centre, Dar Es Salaam, Tanzania
⁶ UCSF Center for Tuberculosis, University of San Francisco, San Francisco, California, USA
⁷ School of Medicine, University of St Andrews, Scotland, UK.
⁸ Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA

# Corresponding author,

Dr. Peter Mbelele
Kibong’oto Infectious Diseases Hospital (KIDH),
P.O BOX 12, Siha, Kilimanjaro, Tanzania
Email: mbelelepeter@yahoo.com

Running title: Monitoring MDR-TB treatment response by TB-MBLA
Abstract

**Background:** Rifampicin or multidrug-resistant-tuberculosis (RR/MDR-TB) treatment has largely transitioned to regimens free of the injectable aminoglycoside component despite the drug class’ purported bactericidal activity early in treatment. We tested whether *Mycobacterium tuberculosis* (*Mtb*) killing rates measured by molecular bacterial load assay (TB-MBLA) in sputa correlate with composition of the RR/MDR-TB regimen.

**Methods:** Serial sputa were collected from patients with RR/MDR- and drug-sensitive TB at day 0, 3, 7, 14, and then monthly for 4 months of anti-TB treatment. TB-MBLA was used to quantify viable *Mtb* 16S rRNA in sputum for estimation of colony-forming-unit per mL (eCFU/mL). *Mtb* killing rates were compared among regimens using nonlinear-mixed-effects modelling of repeated measures.

**Results:** Thirty-seven patients produced 296 serial sputa: 13 patients received an injectable-containing but bedaquiline-free reference regimen, 9 received an injectable and bedaquiline-containing regimen, 8 received an all-oral bedaquiline-based regimen, and 7 patients were treated for drug-sensitive TB with conventional rifampin/isoniazid/pyrazinamide/ethambutol (RHZE). Compared to the adjusted *Mtb* killing of -0.17 (95% CI; -0.23 to -0.12) for the injectable-containing but bedaquiline-free reference regimen, the killing rates were -0.62 (95% CI; -1.05 to -0.20) log_{10} eCFU/mL for the injectable and bedaquiline-containing regimen (p = 0.019), -0.35 (95% CI; -0.65 to -0.13) log_{10} eCFU/mL for the all-oral bedaquiline-based regimen (p = 0.054), and -0.29 (95% CI; -0.78 to +0.22) log_{10} eCFU/mL for RHZE (p = 0.332).

**Conclusion:** *Mtb* killing rates from sputa were higher among patients who received bedaquiline but were further improved with the addition of an injectable aminoglycoside.
Introduction

Measurement of pulmonary tuberculosis (PTB) treatment response in endemic settings largely depends on sputum smear microscopy (1). While the sputum smear microscopy detection threshold is at least $10^3$ *Mycobacterium tuberculosis* (*Mt*) colony-forming-units in 1 mL (CFU/mL) of sputum sample, many patients with PTB such as those with human immunodeficiency virus and the acquired immunodeficiency syndrome (HIV/AIDS) present with paucibacillary disease and may be unable to produce a good quality sputa for detection of acid-fast-bacilli (AFB) (2, 3). Besides, microscopy does not distinguish viable from non-viable *Mt* and therefore does not inform how patients with rifampicin and or multidrug resistant (RR/MDR)-TB respond to treatment (3). Patients with RR/MDR-TB are typically monitored for cultured growth in Lowenstein-Jensen (LJ) solid medium or the Mycobacterium Growth Indicator Tube liquid culture system. Culture is sensitive with a detection limit of 10 – 100 CFU/mL of sputum, yet it is also prone to contamination and can take up to 8 weeks to determine a definitive positive or negative result, thereby limiting the ability to take appropriate and timely clinical action (4).

The deoxyribose nucleic acid (DNA) based methods such as Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and line probe assays (LPA) like genotype MTBDRplus/sl (Hain Lifescience GmbH, Nehren, Germany) are rapid in detecting *Mt* compared to culture-based methods (5, 6). Nevertheless, DNA of *Mt* can persist in a patient’s sputa for a prolonged time after successful treatment (7, 8). Even if the sputum sample is pre-treated with propidium monoazide (Biotium Inc., Hayward, California, USA), a chemical substance previously known to bind the DNA of dead bacilli, the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) cannot distinguish viable from non-viable DNA of *Mt* (9, 10). Therefore, DNA-based assays are not suitable for monitoring TB treatment response. Fortunately, there is growing evidence that ribose nucleic acid (RNA) can serve as a surrogate biomarker for microbial viability (11–13), and therefore they can be used for monitoring TB treatment response (14, 15). TB molecular bacterial load assay (TB-MBLA) was first reported in 2011 as a biomarker for drug-sensitive TB treatment response and proved a robust measure in different settings (14, 16). TB-MBLA is a real-time polymerase chain reaction (RT-qPCR) assay which detects and quantifies killing of 16S rRNA from both viable replicating and dormant *Mt* in patient’s sputa during treatment (14). It is rapid and results are available within 24 hours in time to inform clinical assessment of a patient’s progress (14, 17). TB-MBLA was found to be consistently read as positive for samples with as low as 10 CFU/mL of *Mt*. This bacterial load corresponds to a RT-qPCR quantification cycle (Cq) value of 30, the limit of quantification for positive *Mt* (14). Therefore, TB-MBLA has the potential to replace or compliment both smear microscopy and culture for monitoring TB treatment response (14, 16, 18).
Because the treatment is difficult and complex, more than 40% of patients treated for R/MDR-TB in 2019 worldwide had an unfavorable treatment outcome (19). However, the 10-year RR/MDR-TB treatment success, defined as the total number of patients who achieve microbiological cure and those who complete the treatment regimen, has been consistently above 75% in Tanzania (20). During this decade, the injectable aminoglycoside class of antibiotic such as kanamycin has been one of the backbones of RR/MDR-TB treatment regimens (21). Because administering injectable aminoglycoside is not only invasive to patients but also is associated with severe adverse events including ototoxicity and nephrotoxicity (22, 23), the WHO has endorsed a transition from injectable to all-oral MDR-TB treatment regimens (24). To align with this transition, TB-endemic countries including Tanzania, have recently adopted new and repurposed TB medicines, such as bedaquiline, delamanid and linezolid, and constructed regimens with limited microbiological evidence of effectiveness in patients with RR/MDR-TB. Hence, we deployed TB-MBLA to describe killing of *Mtb* in patients receiving RR/MDR-TB and DS-TB treatment. We tested the hypothesis that *Mtb* killing rates from the sputa, as measured by TB-MBLA, not only correlated with time-to-culture conversion but were dependent upon the composition of the RR/MDR-TB antibiotic regimen.

**Materials and Methods**

**Patients and ethical considerations**

From August 2018 to December 2019, two population of patients with TB participated in this study. The primary target population was patients with RR/MDR-TB. A small population of patients with DS-TB was included as a control to inform the reproducibility of previous TB-MBLA study findings (14, 16). Rifampicin susceptibility in *Mtb* was confirmed using Xpert® MTB/Rif (25). Moreover, all patients harboured *Mtb* that was deemed susceptible to fluoroquinolones and aminoglycosides by line probe assay (Hain, LifeScience, Germany), the genotype MTBDRsl version 2.0 (26). The study included all patients aged at least 18 years and who were able to expectorate and provide quality early-morning sputum. Quality sputum was defined by an adequate volume of > 5 mL and absence of food particles. No sputum induction was done to patients who were unable to provide quality sputum. Critically ill or moribund patients as previously defined by Robertson et al (27) and pregnant women were excluded. Additionally, patients who interrupted treatment were excluded from the final analysis. Prior to any study procedure, all patients signed a witnessed oral or written informed consent. The study was approved by the National Institute for Medical Research (NIMR) in Tanzania (NIMR/HQ/R.8a/Vol. IX/2662). Permission to conduct the study was granted by authorities of the Kibong’oto Infectious Diseases Hospital (KIDH).
Study design and treatment regimens
This was a longitudinal cohort study design where each patient was followed for 16 weeks of anti-TB treatment. The treatment regimens for patients with RR/MDR-TB included; (i) an injectable-containing but bedaquiline free regimen composed of daily dosed kanamycin (15mg/Kg), levofloxacin (750 mg for patients weighing < 50 Kg and 1000mg if weighed ≥ 50 Kg), pyrazinamide (25mg/Kg), ethionamide (750mg) and cycloserine (750mg), (ii) an all-oral based regimen composed of bedaquiline (400mg daily for 2 weeks, 200mg), linezolid (600mg/day), levofloxacin, pyrazinamide and ethionamide, (iii) an injectable and bedaquiline containing regimen composed of kanamycin, bedaquiline, levofloxacin, pyrazinamide and ethionamide; and (iv) standard fixed-dose combination containing rifampicin (150mg), isoniazid (75mg), pyrazinamide (400mg) and ethambutol (275 mg), termed as RHZE, for patients with DS-TB. Patients weighing < 50 kg received three tablets of and those weighing ≥ 50 kg received four tablets of RHZE per day.

Study Setting
Patients were recruited at Kibong’oto Infectious Diseases Hospital, a national centre of excellence for clinical management of drug resistant (DR)-TB located in Siha district of Kilimanjaro region in Tanzania (25). TB-MBLA testing was performed at the National Institute for Medical Research, Mbeya Medical Research Centre branch, given that laboratory’s prior experience with the assay.

Sample size determination
The numbers of patients required to determine differences in bactericidal activity over time in 4 treatment regimens were calculated as previously reported by Guo et al (28). We assumed a Spearman correlation of 0.51, and a baseline Mtb burden of 5.5 log₁₀ eCFU/mL, as well as daily Mtb decline and decay rate of 0.42 and 0.05 log₁₀ eCFU/mL respectively (14, 16). Hence, at least 7 patients were needed per regimen to reach a power of 80% with a two-sided type I error of 5%. Adjusting for least 25% of patients who were likely to be lost to follow up, not evaluated due to negative microbiological results at baseline and or died, a minimum of 37 patients were desirable to be sampled and analysed at the end of 4 months treatment follow up.

Sputum collection, processing and culturing of M. tuberculosis
One sample of approximately 5 mLs of early morning sputum was collected from each patient for laboratory testing at day 0 (baseline), 3, 7, 14, 28, 56, 84 and 112 of anti-TB treatment. Before culturing, sputum was homogenized using a sterile magnetic stirrer at room temperature for 30 minutes. Then, 1mL of homogenized sputum was treated using 4 mLs of 4M guanidine thiocyanate (GTC) containing of 1 M Tris–HCl (pH 7.5) and 1% (V/V) of β-mercaptoethanol, and was frozen at −80°C in order to preserve the Mtb RNA from degrading enzymes. The Mtb culture was performed on LJ slants from the remaining sputum samples collected at six time points from day 0, 14, 28, 56, 84
and 112 of treatment as per previous instructions (29). In brief, sputum was decontaminated by NALC-NaOH, and finally re-suspended in 1 mL of phosphate buffer. A total of 200 μL of decontaminated sputum was inoculated in two LJ slants and incubated for up to 8 weeks to detect mycobacteria growth. Incubated LJ slants were read on weekly basis and were deemed negative if no growth at week 8.

**RNA extraction and RT-qPCR for TB-MBLA**

*Mtb* quantification by TB-MBLA was performed as described by Gillespie et al (30). In summary, *Mtb* RNA in 1mL of homogenized sputum preserved in 4 mLs of guanidine thiocyanate (GTC) at −80°C was extracted using the RNA pro (FastRNA Pro BlueKit; MP Biomedical, California, USA) as instructed by manufacturer. The extract was treated with DNase I enzyme (TURBO DNA-Free Kit; Ambion, California, USA) to remove DNA from the dead cells. The *Mtb* 16S rRNA, a biomarker for viable cells, was amplified and quantified by RT-qPCR using specific primers and probes. The Cq was translated to bacterial load (estimated CFU per mL (eCFU/mL) using a standard curve on a Rotor gene Q 5plex platform (Qiagen). The cut-off for TB-MBLA positivity is a 30 Cq value that corresponds to 1.0 log_{10} eCFU/mL, beyond which the test was considered negative (16, 30).

**Statistical analysis**

Data were recorded in a clinical case report form, entered and cleaned before statistical analysis. Patients who completed 8 treatment visits and had positive pre-treatment TB-MBLA results were analysed and visualised in R, version 4.0.2 (http://www.R-project.org). Continuous variables such as age, body-mass-index (BMI) in Kg/m² and time to TB-MBLA negativity were described as median with their 25th and 75th interquartile range (IQR), and were compared across different regimens using a Kruskal–Wallis test. Accordingly, proportions for HIV status, gender, cavitary-disease on chest and previous TB treatment were compared across different regimens using Chi-Square or Fischer’s exact test. Using baseline bacterial load, chest cavity, HIV, silicosis and gender as fixed effects, the rate of *Mtb* killing (log_{10}CFU/mL) was fitted on quartic polynomial nonlinear-mixed-effects (NLME) for repeated measures as previous (31, 32). Individual patients were accounted for random effect. A model was reliably selected if it had low Akaike-information-criterion but high intraclass-correlation-coefficient (Table 1). The mean difference in *Mtb* load, due to two different regimens received by patients at the end of 4 months of treatment, was compared using one-way analysis-of-variance (ANOVA) and Tukey’s test for repeated measures (33). An injectable containing regimen without bedaquiline was used as a reference regimen. The median time to TB-MBLA and culture conversion to negative was estimated using the Kaplan-Meier method, and was compared across different regimens using a log-rank test (34). Cox Proportional-Hazards regression models were used to estimate the hazard ratios (HR) for *Mtb* killing, and was adjusted for the effects of HIV, baseline
bacillary load, cavitary disease, silicosis, gender, prior history of treatment for drug sensitive TB and
clearance rate. We computed an overall mean *Mtb* load of 4.0 \( \log_{10} \) eCFU/mL, and it was used to
categorize a patient’s bacterial load as “high bacterial load” and “low bacterial load” if patients had
detectable *Mtb* above and below this mean respectively. A \( p \) value < 0.05 was considered significance.
A 95% confidence interval (CI) of the mean clearance rate and HR was included.

**Results**

**Population**

Of 59 patients enrolled, 37 patients produced a total of 296 serial sputa for final analysis. Reasons for
exclusion and patient’s distribution are outlined in Figure 1. Among 296 serial sputa analysed, 104
sputa came from 13 patients who received an injectable-containing but bedaquiline-free regimen, 72
sputa from 9 patients who received an injectable and bedaquiline-containing regimen, 64 from 8
patients who received an all-oral bedaquiline-based regimen, and 56 sputa from 7 patients who were
treated for drug-sensitive TB with conventional RHZE. Clinical and demographics are presented in
Table 2. Twenty-seven (73%) out of 37 patients were male. Their median (IQR) age was 37 (32 – 49)
years. Patients who received standard RHZE treatment were younger than those who received
RR/MDR-TB treatment regimens \( (p = 0.038) \). Also, 11 (30%) patients were living with HIV with a
CD4 T cell count of 208 (95% CI; 144 – 272) cells/µL. More patients with HIV received an all-oral
than injectable-based treatment regimen \( (p = 0.001) \).

**Mycobactericidal activities of different regimens over time**

The *Mtb* load measured by TB-MBLA and culture in Figure 2 decreased significantly over time \( (R =
-0.77, p < 0.001) \). The mean *Mtb* load in \( \log_{10} \) eCFU/mL (95% CI) was reduced from 5.19 (4.40 –
5.78) at baseline to 3.10 (2.70 – 3.50) at day 14, then to 2.52 (2.13 – 2.90) at day 28, 1.88 (1.53 -2.22)
at day 56 and <1.36 (1.03 – 1.70) at day 84 through 112 of treatment. The overall mean daily *Mtb*
 killed was -0.24 (95% CI; -0.39 to -0.08) \( \log_{10} \) eCFU/mL, and it varied with treatment-regimen
(Table 3, \( p < 0.001 \)). An injectable and bedaquiline containing regimen had the highest mean *Mtb*
 killing rate followed by an all-oral bedaquiline based regimen compared to injectable-containing but
bedaquiline free reference regimen (Table 3, \( p = 0.019 \)). Kanamycin containing regimens in Figure 3
had rapid bactericidal activity at day 14, but this was not translated into long term bactericidal effect
\( (p < 0.001) \). An all-oral bedaquiline-based regimen had a sharp decline after day 28.

**Median time to *M. tuberculosis* killing**

There was strong positive correlation in time-to sputum conversion between TB-MBLA and culture \( [r
= 0.46 (95% CI; 0.36 – 0.55), p < 0.001] \). The overall median time to sputum TB-MBLA conversion
to negative was 56 (IQR; 28-84) days. The median time to TB-MBLA conversion to negative were
28, 42 and 84 days among patients on injectable and bedaquiline, an all-oral bedaquiline-based regimen, and injectable-containing but bedaquiline free regimens respectively. Irrespective of treatment regimen, 92% (34/37) of patients had negative culture results compared to 65% (24/37) of negative TB-MBLA at day 56 (p = 0.037). The number of patients who converted to sputum negative by culture and TB-MBLA per treatment regimen are shown in Figure 4. Among 13 patients who received injectable but bedaquiline free containing regimen, 2 and 7 of them remained culture and TB-MBLA positive respectively, whereas all 8 patients who received injectable and bedaquiline containing regimens had negative LJ culture and TB-MBLA at day 56 (Figure 4 A, B, C & D). Favourably, all patients on injectable-bedaquiline MDR-TB and standard RHZE regimen for DS-TB had negative TB-MBLA at day 56 and 84 respectively. Compared to 31% (4/13) of patients who received an injectable but bedaquiline free regimen, only 11% (1/9) of those who received an all-oral bedaquiline containing regimen remained positive TB-MBLA but negative LJ culture at day 112 of treatment (Figure 4 A & B vs. Figure 4 E & F, p = 0.283).

**Hazard ratio (HR) of M. tuberculosis killing**

The overall mean $Mtb$ load $\log_{10}$ eCFU/mL at baseline was 5.19 (95% CI; 4.40 – 5.78), and was similar in all patients treated with any of the 4 regimens (Table 3, p = 0.453). The mean $Mtb$ load ($\log_{10}$ eCFU/mL) among female was 5.6 (95% CI; 5.0 – 6.2) $\log_{10}$ eCFU/mL compared to 4.7 (95% CI; 4.3 – 5.2) $\log_{10}$ eCFU/mL among male (p = 0.017) patients. Patients with chest cavity had mean $Mtb$ load of 5.26 (95% CI; 4.45 – 5.87) compared to 4.40 (95% CI; 3.91 – 4.75) $\log_{10}$ eCFU/mL in those without cavity (p = 0.080). Adjusting for bacterial load, initial killing rate, silicosis, chest cavity, HIV and gender, the hazard-ratios for $Mtb$ killing were 12.37 (95% CI, 2.87 – 53.30; p = 0.001) and 14.31 (95% CI, 3.49 – 58.65; p < 0.001) for patients who received an all-oral bedaquiline and injectable and bedaquiline-containing regimens respectively (Table 4). Bacterial load at baseline strongly correlated positively with median time to sputum conversion to negative by both TB-MBLA and culture $[r = 0.48 (95\% CI; 0.18 – 0.69), p = 0.003]$. High $Mtb$ load and TB/silicosis were independently predictor of slow $Mtb$ killing compared to low $Mtb$ load and TB without silicosis (Table 4, p ≤ 0.033).

**Discussion**

This study shows for the first time to our knowledge that the killing rates of $Mtb$ in patients treated for RR/MDR-TB as well as those with concomitant TB/silicosis varies with treatment regimens. As measured by TB-MBLA, $Mtb$ decreased significantly over time on treatment, and this kinetic correlated with what was observed using LJ culture medium. For decades, culture has been used as a routine microbiological tool for monitoring drug-resistant TB treatment response (15, 35), but in many TB endemic settings, culture is unavailable or limited to specialized centres. Importantly, culture
results can take up to 8 weeks from the time of sputum collection, which delays patient care if a treatment decision is made based on a result from a specimen collected two months earlier. Given the continued decentralization of RR/MDR-TB services in Tanzania and elsewhere, monitoring treatment response in laboratories capable of performing qPCR, such as with Xpert MTB/RIF, will allow laboratory assays to impact treatment decisions closer to the point-of-care. Therefore this study in RR/MDR-TB compliments the growing evidence base for the application of TB-MBLA in routine clinical management (14, 16, 36).

Interestingly, our findings suggest that bactericidal activity at day 14 may not be a suitable predictor of the long-term efficacy of a regimen, particularly when that regimen is bedaquiline containing. In this cohort at day 14, more than 75% of people had a positive TB-MBLA and more than half had a positive culture result. Whereas between 14-56 days we observed substantial \textit{Mtb} killing in those treated with a bedaquiline containing regimens, suggesting that evaluation of bactericidal activity be performed later, such as at day 56, for modern RR/MDR-TB regimens. Using culture, one previous phase 2b clinical trial reported high bactericidal activity of a bedaquiline containing regimen in patients with DS- and RR/MDR-TB (37). However, detectable \textit{Mtb} beyond day 56 in our study supports this trial’s argument that day 56 is unreliable indicator of a regimen’s ability to either predict long term treatment outcomes or shorten treatment duration (37). This further raises the question of whether TB-MBLA may in fact be a superior predictor to culture.

Another important finding from this study of TB-MBLA is that \textit{Mtb} killing kinetics were regimen-dependent. Overall, there was rapid and prominent killing of \textit{Mtb} at day 14 for patients who received kanamycin regardless of receipt of bedaquiline. However, superior activity of kanamycin containing regimens at day 14 had no long term-bactericidal effect. As a result, 3 patients on injectable containing but bedaquiline free regimen remained positive by TB-MBLA but negative by LJ culture after 4 months of treatment. On the other hand, patients who received an all-oral bedaquiline containing regimen achieved these rates of killing at or after 1 month of treatment. This observation concurs with previous reports that the bactericidal activity of bedaquiline in MDR-TB is delayed at the beginning, but accelerates later in therapy (38). Usually, recovery of \textit{Mtb} by TB-MBLA correlates better with MGIT liquid than LJ-culture, which may partially explain the discrepancy between the two tests at month 4 of treatment (16). This argument supports previous findings that culturing \textit{Mtb} on LJ recovers a lower yield than in MGIT liquid culture (39). Nonetheless, our findings as measured by TB-MBLA fit with the pharmacodynamical understanding that kanamycin and other aminoglycoside/polypeptides if active against mycobacteria, primarily exert their effect against those extracellular organisms that are rapidly dividing and may be more abundant early in the treatment course (40, 41).
The shorter overall time to sputum conversion to negative, as measured by TB-MBLA and conventional culture, for all patients who received bedaquiline regardless of kanamycin further supports arguments that bedaquiline should be a cornerstone of regimens designed to shorten MDR-TB treatment duration (42). The conventional injectable-containing but bedaquiline free regimen has been in practice for decades, even though more than 40% of patients treated with this regimen had unfavourable outcomes in TB endemic settings (43). Aminoglycosides such as kanamycin are no longer part of the current MDR-TB treatment regimens not because of its lack of bactericidal activity, as our data would suggest the contrary in the early treatment period, but rather because of the significant toxicity and patient intolerances that led to treatment interruption (24, 44). From a microbiological perspective alone, as demonstrated in this study and others such as Mpagama et al. (45), and in a more patient-centred approach however, our results demonstrate the potential importance of finding a tolerable substitutes for kanamycin that can match the early bactericidal effect.

The main strengths in this study are that we have utilized TB-MBLA to model killing rates among patients with RR/MDR-TB and those with TB/silicosis. We have shown that patients with TB/silicosis had slower \textit{Mtb} killing rates by TB-MBLA compared to those with TB and without silicosis. This slow rate of killing could partially be attributed to the underlying pulmonary pathophysiology which can include progressive massive fibrosis (46, 47), and a blunted local host immune response to \textit{Mtb} infection (46). We observed a similarly slower rate of \textit{Mtb} killing among patients with RR/MDR-TB who had high initial bacterial load, which supplements previous studies of TB-MBLA kinetics from patients with drug sensitive TB (14, 16, 36). In this study, approximately 1 and 4 out of 10 patients had respectively positive LJ-culture and TB-MBLA at day 56. This supports the previous argument that TB-MBLA is more sensitive compared to agar based Loewenstein Jensen culture, in which \textit{Mtb} population gets lost due to decontamination procedures in the later (18). Limitations of the study include the timing of endpoints which were limited to 4 months such that predicting long-term treatment success was beyond the scope of this study. Nevertheless, modelling \textit{Mtb} killing for 4 months as we accomplished here has been used as marker for treatment failure and relapse in several observational studies (35, 48), and exceeds the duration of monitoring used in other trials of RR/MDR-TB regimens that have employed conventional culture based techniques. (37) Additionally, this study had no control over the treatment regimens prescribed. However, given the feasibility of TB-MBLA and the comparability of this study’s findings to those prior with TB-MBLA in drug-susceptible TB (16), we plan to apply TB-MBLA systematically within an ongoing operational research protocol for injectable-free RR/MDR-TB treatment in Tanzania, that employs standardized regimens over varying treatment durations. Lastly, because of the small number of patients per treatment regimen, these findings should be cautiously inferred to other RR/MDR-TB populations.
Nevertheless, a longitudinal cohort design in this study allowed control of variabilities between patients and as well as tracking within-person regimen’s bactericidal activities over time (28, 49).

In conclusion, patients who received bedaquiline-containing regimens exhibited higher \textit{Mtb} killing-rates and had shorter time to sputum TB-MBLA and culture conversion to negative. While both kanamycin containing regimens had superior bactericidal activity during the first two weeks of RR/MDR-TB treatment, the addition of bedaquiline allowed for improved killing after 1 month of therapy. Together, these findings provide insight into formulating optimal all-oral bedaquiline containing regimens with the best potential to shorten MDR-TB treatment duration (37, 44, 50). Given the fact that TB-MBLA does not require laboratory procedures associated with culture and the prolonged time to receive a culture-based result, we envision that it can be used to make regimen adjustments in presence of anti-TB drug susceptibility testing results.

Acknowledgements

This study received financial support from the EDCTP2 programme supported by the European Union project (grant number: TMA2016SF-1463-REMODELTZ) and DELTAS Africa Initiative (Afrique One-ASPIRE /DEL-15-008). The Afrique One-ASPIRE is funded by a consortium of donors including the African Academy of Sciences, Alliance for Accelerating Excellence in Science in Africa, the New Partnership for Africa’s Development Planning and Coordinating Agency, the Wellcome Trust (107753/A/15/Z), and the UK Government. All funding bodies have had no role in the conceptualization, methodology, data interpretation and writing of manuscript.

Furthermore, authors acknowledge Ms Batuli Mono, Taji Mnzava, Joseph Kachala and Dr Bibie Said of KIDH for their assistant with recruitment and data collection from study participants. We also thank Mr. Elisha S. Juma and Ms Sarapia P. Malya of KIDH, and Emmanuel Sichone and Joseph John of NIMR Mbeya for assisting with laboratory work. In addition, we also acknowledge the KIDH administration for granting permission to conduct this study.

Transparency declarations.

All authors have no conflict of interest to declare. PMM, EAM, ES, WS and SGM conceived the study, designed the work and interpreted clinical and TB-MBLA results. PMM and BM acquired data. PMM, KK, EAM, PPJP, WS, and SGM analyzed the data. PMM drafted the manuscript and responded to all co-authors’ inputs. SHG, NEN, and SKH reviewed the manuscript. All authors wrote, approved and agreed to be accountable for all scientific aspects in the final version of this manuscript.

References

1. Mitnick CD, White RA, Lu C, Rodriguez CA, Bayona J, Becerra MC, Burgos M, Centis R,
Cohen T, Cox H, D’Ambrosio L, Danilovitz M, Falzon D, Gelmanova IY, Gler MT, Grinsdale JA, Holtz TH, Keshavjee S, Leimane V, Menzies D, Migliori GB, Milstein MB, Mishustin SP, Pagano M, Quelapio MI, Shean K, Shin SS, Tolman AW, Van Der Walt ML, Van Deun A, Viiklepp P, Ahuja SD, Andreev YG, Ashkin D, Avendano M, Banerjee R, Bauer M, Benedetti A, Brand J, Chan ED, Chiang CY, DeRiemer K, Dung NH, Enarson D, Flanagan K, Flood J, García-García L, Gandhi N, Granich RM, Hollm-Delgado MG, Iseman MD, Jarlsberg LG, Kim HR, Koh WJ, Lancaster J, Lange C, De Lange WCM, Leung CC, Li J, Maug AKJ, Narita M, Odendaal R, O’Riordan P, Pai M, Palmero D, Seung-Kyu, Pasvol G, Pérez-Guzmán C, Ponce-De-Leon A, Riekstina V, Robert J, Royce S, Schaaf HS, Seung KJ, Shah L, Shim TS, Shiraishi Y, Sifuentes-Osornio J, Strand MJ, Shaheed PT, Tupasi TE, Van Altena R, Van Der Werf TS, Vargas MH, Westenhouse J, Yew WW, Yim JJ. 2016. Multidrug-resistant tuberculosis treatment failure detection depends on monitoring interval and microbiological method. Eur Respir J 48:1160–1170.

2. Park JH, Choe J, Bae M, Choi S, Jung KH, Kim MJ, Chong YP, Lee SO, Choi SH, Kim YS, Woo JH, Jo KW, Shim TS, Kim MY, Kim SH. 2019. Clinical characteristics and radiologic features of immunocompromised patients with pauci-bacillary pulmonary tuberculosis receiving delayed diagnosis and treatment. Open Forum Infect Dis 6:1–9.

3. Prasanta Kumar Das, Somtirtha B. Ganguly BMS. 2019. Sputum Smear Microscopy in Tuberculosis: It Is Still Relevant in the Era of Molecular Diagnosis When Seen from the Public Health Perspective. Biomed Biotechnol Res J 3:77–9.

4. van Zyl-Smit RN, Binder A, Meldau R, Mishra H, Semple PL, Theron G, Peter J, Whitelaw A, Sharma SK, Warren R, Bateman ED, Dheda K. 2011. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. PLoS One 6:e28815.

5. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiiyev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377:1495–505.

6. World Health Organization. 2016. The Use of Molecular Line Probe assays for detection of resistance to second line anti-tuberculosis drugs: Policy Guidance. 20 Avenue Appia, 1211 Geneva 27, Switzerland.

7. Nicol MP. 2013. Xpert MTB/RIF: Monitoring response to tuberculosis treatment. Lancet Respir Med 1:427–428.
8. Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, Chanda D, Esmail A, Peter J, Dheda K. 2016. Xpert MTB/RIF Results in Patients With Previous Tuberculosis: Can We Distinguish True From False Positive Results? Clin Infect Dis 62:995–1001.

9. Vladyslav Nikolayevskyy, Paolo Miotto, Edita Pimkina, Yanina Balabanova, Irina Kontsevaya, Olga Ignatyeva, Alessandro Ambrosi, Girts Skenders, Arvydas Ambrozaitis, Alexander Kovalyov, Anna Sadykhova, Tatiana Simak, Andrey Kritsky, Svetlana Mironova, Olesya FD. 2014. Utility of propidium monoazide viability assay as a biomarker for a tuberculosis disease. Tuberculosis 95:179–185.

10. Kayigire XA, Friedrich SO, Karinja MN, van der Merwe L, Martinson NA DA. 2016. Propidium monoazide and Xpert MTB/RIF to quantify Mycobacterium tuberculosis cells. Tuberculosis 110:79–84.

11. Cangelosi GA, Meschke JS. 2014. Dead or alive: Molecular assessment of microbial viability. Appl Environ Microbiol 80:5884–5891.

12. Li R, Tun HM, Jahan M, Zhang Z, Kumar A, Fernando D, Farenhorst A, Khafipour E. 2017. Comparison of DNA-, PMA-, and RNA-based 16S rRNA Illumina sequencing for detection of live bacteria in water. Sci Rep 7:5752.

13. Desjardin LE, Perkins MD, Wolski K, Haun S, Teixeira L, Chen Y, Johnson JL, Ellner JJ, Dietze R, Bates J, Cave MD, Eisenach KD. 1999. Measurement of sputum Mycobacterium tuberculosis messenger RNA as a surrogate for response to chemotherapy. Int J Lepr Other Mycobact Dis 67:521.

14. Honeyborne I, McHugh TD, Phillips PPJ, Bannoo S, Bateson A, Carroll N, Perrin FM, Ronacher K, Wright L, Van Helden PD, Walzl G, Gillespie SH. 2011. Molecular bacterial load assay, a culture-free biomarker for rapid and accurate quantification of sputum Mycobacterium tuberculosis bacillary load during treatment. J Clin Microbiol 49:3905–3911.

15. Rockwood N, du Bruyn E, Morris T, Wilkinson RJ. 2016. Assessment of treatment response in tuberculosis. Expert Rev Respir Med 10:643–654.

16. Sabiiti W, Azam K, Farmer ECW, Kuchaka D, Mtafya B, Bowness R, Oravcova K, Honeyborne I, Evangelopoulos D, McHugh TD, Khosa C, Rachow A, Heinrich N, Kampilra E, Davies G, Bhatt N, Ntinginya EN, Viegas S, Jani I, Kamdolozi M, Mdolo A, Khonga M, Boeree MJ, Phillips PPJ, Sloan D, Hoelscher M, Kibiki G, Gillespie SH. 2020. Tuberculosis bacillary load, an early marker of disease severity: The utility of tuberculosis Molecular Bacterial Load Assay. Thorax 0:1–3.

17. Shiferaw MB, Yismaw G. 2019. Magnitude of delayed turnaround time of laboratory results in...
18. Mtafya B, Sabiiti W, Sabi I, John J, Sichone E, Ntinginya NE, Gillespie SH. 2019. Molecular bacterial load assay concurs with culture on NaOH-induced loss of mycobacterium tuberculosis viability. J Clin Microbiol 57:1–9.

19. World Health Organization. 2020. Global tuberculosis report 2020. Geneva, Switzerland.

20. Leveri TH, Lekule I, Molloe E, Lyamuya F, Kilonzo K. 2019. Predictors of Treatment Outcomes among Multidrug Resistant Tuberculosis Patients in Tanzania. Tuberc Res Treat 2019:1–10.

21. WHO. 2014. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. World Health Organization.

22. Harris T, Bardien S, Simon Schaaf H, Petersen L, de Jong G, Fagan JJ. 2016. Aminoglycoside-induced hearing loss in HIV-positive and HIV-negative multidrug-resistant tuberculosis patients. South African Med J 102:363–366.

23. Perumal R, Abdelghani N, Naidu N, Yende-Zuma N, Dawood H, Naidoo K, Singh N, Padayatchi N. 2018. Risk of nephrotoxicity in patients with drug-resistant tuberculosis treated with Kanamycin/capreomycin with or without concomitant use of tenofovir-containing antiretroviral therapy. J Acquir Immune Defic Syndr 78:536–542.

24. World Health Organization. 2019. WHO Consolidated guidelines on drug-resistant tuberculosis treatment. World Heal Organ. World Health Organization, Geneva, Switzerland.

25. Mbelele PM, Aboud S, Mpagama SG, Matee MI. 2017. Improved performance of Xpert MTB/RIF assay on sputum sediment samples obtained from presumptive pulmonary tuberculosis cases at Kibong’oto infectious diseases hospital in Tanzania. BMC Infect Dis 17:1–7.

26. Theron G, Peter J, Richardson M, Warren R, Dheda K, Steingart KR. 2016. GenoType® MTBDR sl assay for resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev https://doi.org/10.1002/14651858.CD010705.pub3.

27. Robertson LC, Al-Haddad M. 2013. Recognizing the critically ill patient. Anaesth Intensive Care Med 14:11–14.

28. Guo Y, Logan HL, Glueck DH, Muller KE. 2013. Selecting a sample size for studies with repeated measures. BMC Med Res Methodol 13:100.

29. Tripathi K, Tripathi PC, Nema S, Shrivastava AK, Dwivedi K. 2014. Modified Petroff’s Method: an Excellent Simplified Decontamination Technique in Comparison with Petroff’s
Method. Int J Recent Trends Sci Technol 10:461–464.

30. Gillespie H Stephen SW and OK. 2017. Mybacterial Load Assay. Methods Mol Biol 1616:155–170.

31. Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, Reddy C, Sturm AW, Sirgel FA, Allen J, Coleman DJ, Fourie B, Mitchison DA, Bah-Sow OY, Diop H, Fielding K, Gninafon M, Mitchison D, Lienhardt C, Odhiambo J, Perronne C, Portaels F, Rustomjee R, Ramjee A, Master I, Olowolagba A, Chinappa T, Osburne G, Bamber S, Pala AS, Pillay L, Tembe C, Mpangase P, Hadebe T, Ngcobo CP, Mkhize Z, Dlamini CN, Gill L, Dube T, Saul M, Merle C, Suma KF. 2008. A phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis 12:128–138.

32. Movshovitz-Hadar N, Shmukler A. 1991. A qualitative study of polynomials in high school. Int J Math Educ Sci Technol 22:523–543.

33. Hazra A, Gogtay N. 2016. Biostatistics series module 3: Comparing groups: Numerical variables. Indian J Dermatol 61:251–260.

34. Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, Pappas F, Phillips PPJ, Nunn AJ. 2014. Four-month Moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med 371:1577–1587.

35. Goletti D, Lindestam Arlehamn CS, Scriba TJ, Anthony R, Cirillo DM, Alonzi T, Denkinger CM, Cobelens F. 2018. Can we predict tuberculosis cure? What tools are available? Eur Respir J 52.

36. Honeyborne I, Mtafya B, Phillips PPJ, Hoelscher M, Ntinginya EN, Kohlenberg A, Rachow A, Rojas-Ponce G, McHugh TD, Heinrich N. 2014. The molecular bacterial load assay replaces solid culture for measuring early bactericidal response to antituberculosis treatment. J Clin Microbiol 52:3064–3067.

37. Tweed CD, Dawson R, Burger DA, Conradie A, Crook AM, Mendel CM, Conradie F, Diacon AH, Ntinginya NE, Everitt DE, Haraka F, Li M, van Niekerk CH, Okwera A, Rassool MS, Reither K, Sebe MA, Staples S, Variava E, Spigelman M. 2019. Bedaquiline, moxifloxacin, pretomanid, and pyrazinamide during the first 8 weeks of treatment of patients with drug-susceptible or drug-resistant pulmonary tuberculosis: a multicentre, open-label, partially randomised, phase 2b trial. Lancet Respir Med 7:1048–1058.

38. Nguyen TVA, Cao TBT, Akkerman OW, Tiberi S, Vu DH, Alffenaar JWC. 2016. Bedaquiline as part of combination therapy in adults with pulmonary multi-drug resistant tuberculosis. Expert Rev Clin Pharmacol 9:1025–1037.
39. Diriba G, Kebede A, Yaregal Z, Getahun M, Tadesse M, Meaza A, Dagne Z, Moga S, Dilebo J, Gudena K, Hassen M, Desta K. 2017. Performance of Mycobacterium Growth Indicator Tube BACTEC 960 with Lowenstein – Jensen method for diagnosis of Mycobacterium tuberculosis at Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia. BMC Res Notes 10:1–6.

40. Krause KM, Serio AW, Kane TR, Connolly LE. 2016. Aminoglycosides: An overview. Cold Spring Harb Perspect Med 6:1–18.

41. Motta I, Calcagno A, Bonora S. 2018. Pharmacokinetics and pharmacogenetics of anti-tubercular drugs: a tool for treatment optimization? Expert Opin Drug Metab Toxicol 14:59–82.

42. Doan TN, Cao P, Emeto TI, McCaw JM, McBryde ES. 2018. Predicting the outcomes of new short-course regimens for multidrug-resistant tuberculosis using intrahost and pharmacokinetic-pharmacodynamic modeling. Antimicrob Agents Chemother 62:1–11.

43. World Health Organization. 2019. Global tuberculosis report 2019. Geneva: World Health Organization.

44. World Health Organization. 2018. Rapid Communication: Key changes to treatment of multidrug- and rifampicin-resistant tuberculosis. World Health Organisation. Geneva.

45. Mpagama SG, Ndusilo N, Stroup S, Kumburu H, Peloquin CA, Gratz J, Houpt ER, Kibiki GS, Heysell SK. 2014. Plasma Drug Activity in Patients on Treatment for Multidrug- Resistant Tuberculosis: Antimicrob Agents Chemother 58:782–788.

46. Konečný P, Ehrlich R, Gulumian M, Jacobs M. 2019. Immunity to the dual threat of silica exposure and mycobacterium tuberculosis. Front Immunol 9:3069.

47. Skowroński M, Halicka A, Barinow-Wojewódzki A. 2018. Pulmonary tuberculosis in a male with silicosis. Adv Respir Med 86:121–125.

48. Ahmad N, Ahuja SD, Akkerman OW, Alfenaar JWC, Anderson LF, Baghaii P, Bang D, Barry PM, Bastos ML, Behera D, Benedetti A, Bisson GP, Boeree MJ, Bonnet M, Brode SK, Brust JCM, Cai Y, Caumes E, Cegielski JP, Centis R, Chan PC, Chan ED, Chang KC, Charles M, Cirule A, Dalcolmo MP, D’Ambrosio L, de Vries G, Dheda K, Esmail A, Flood J, Foissy JG, Fréchet-Jachym M, Fregoni G, Gayoso R, Gegia M, Gler MT, Gu S, Guglielmetti L, Holtz TH, Hughes J, Isaakidis P, Jarlsberg L, Kempker RR, Keshavjee S, Khan FA, Kipiani M, Koenig SP, Koh WJ, Kritski A, Kvasnovsky CL, Kwak N, Lan Z, Lange C, Laniado-Laborín R, Lee M, Leimane V, Leung CC, Leung ECC, Li PZ, Lowenthal P, Maciel EL, Marks SM, Mase S, Mbuagbaw L, Migliori GB, Milanov V, Miller AC, Mitnick CD, Modongo C, Mohr E, Monedero I, Nahid P, Ndjeka N, O’Donnell MR, Padayatchi N, Palmero D, Pape JW, Podewils...
LJ, Reynolds I, Riekstina V, Robert J, Rodriguez M, Seaworth B, Seung KJ, Schnippel K, Shim TS, Singla R, Smith SE, Sotgiu G, Sukhbaatar G, Tabarsi P, Tiberi S, Trajman A, Trieu L, Udwadia ZF, van der Werf TS, Veziris N, Viiklepp P, Vilbrun SC, Walsh K, Westenhouse J, Yew WW, Yim JJ, Zetola NM, Zignol M, Menzies D. 2018. Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet 392:821–834.

Schober P, Vetter TR. 2018. Repeated measures designs and analysis of longitudinal data: If at first you do not succeed-try, try again. Anesth Analg 127:569–575.

Silva DR, Mello FC de Q, Migliori GB. 2020. Shortened tuberculosis treatment regimens: what is new? J Bras Pneumol 46:e20200009.
Figure legends

**Figure 1. Recruitment and patient’s distributions in different treatment regimens.** Patients with drug-sensitive (DS) and rifampicin/multidrug-resistant (RR/MDR)-TB were recruited and treated using different anti-TB treatment regimens. Regimens included standard RHZE comprised of rifampicin, isoniazid, PZA & ethambutol; injectable-bedaquiline (BDQ) free regimen comprised of kanamycin (KAN), levofloxacin (LFX), pyrazinamide (PZA), ethionamide (ETH) and Cycloserine (CS); injectable-BDQ containing regimen comprised of KAN, BDQ, LFX, PZA and ETH; and All-oral BDQ regimen contained BDQ, LFX, linezolid (LZD), PZA and ETH. Among other criteria, 11 out of 59 patients recruited had negative TB molecular bacterial load assay (TB-MBLA) at baseline and were excluded from final analysis.

**Figure 2. M. tuberculosis killing during the first 4 months of anti-TB treatment.** The plots A-F show M. tuberculosis (Mt) kinetics as measured TB-MBLA during treatment with different anti-TB regimens. The dotted line is the cut-off value for positive TB-MBLA test. While Figure 2 A shows overall time-dependent decline of Mt load in estimated colony forming unit per 1 mL (eCFU/mL) of sputum between patients as measured by TB-MBLA, Figure 2B delineates this decline as measure by both TB-MBLA and Lowenstein-Jensen culture. Overall, bacterial load at baseline strongly correlate positively with median time to sputum conversion to negative by both TB-MBLA and culture. Patients with higher bacterial load at baseline had culture conversion later than those with lower. Figure 2 C – F shows Mt decline in eCFU/mL among patients treated with standard RHZE (C); injectable bedaquiline free regimen (D) containing kanamycin (KAN), levofloxacin (LFX), pyrazinamide (PZA), ethionamide (ETH) and Cycloserine (CS); Injectable-bedaquiline regimen (E) was comprised of KAN, Bedaquiline (BDQ), LFX, PZA and ETH; and an all-oral bedaquiline regimen (F) containing BDQ, LFX, linezolid (LZD), PZA and ETH.

**Figure 3. Kaplan-Meier curves showing median time to M. tuberculosis killing in patient’s sputum per treatment regimen.** The dotted lines denote the median time to TB-MBLA conversion from positive to negative Bedaquiline containing regimens had short median time to TB-MBLA conversion to negative compared to injectable-containing but bedaquiline free regimen containing kanamycin (KAN), levofloxacin (LFX), pyrazinamide (PZA), ethionamide (ETH) and Cycloserine (CS). Injectable-bedaquiline was comprised of KAN, bedaquiline (BDQ), LFX, PZA and ETH; an all-oral bedaquiline regimen was composed of BDQ, LFX, linezolid (LZD), PZA and ETH; and Standard RHZE composed of rifampicin, isoniazid, PZA and ethambutol.

**Figure 4. Number of patients who converted to negative by TB-MBLA and Lowenstein-Jensen culture during the first 4 months of treatment with different anti-TB regimens.** The overall
sputum conversion from positive to negative TB-MBLA and LJ culture results had the same trend in four different regimens. At recruitment (day 0), all 37 patients had positive TB-MBLA and culture (MBLA+, LJ+). Both TB-MBLA and culture tests were respectively negative (MBLA-, LJ-) at day 56 and 84 in all patients on injectable plus bedaquiline (B) and those on standard RHZE (D) composed of rifampicin, isoniazid, PZA and ethambutol. A total of 3 patients who received injectable-bedaquiline free (A) compared to 1 patient on all-oral bedaquiline (C) regimen remained TB-MBLA positive but culture negative (MBLA+, LJ-).
Table 1. Fitting and selection of a reliable polynomial nonlinear mixed effects model for repeated measures

| Polynomial models | Intercepts (log10 eCFU/mL) | Intraclass correlation coefficient (ICC) | Standard deviation (SD) | Akaike Information criterion (AIC) | Likelihood ratio test | p value |
|-------------------|-----------------------------|-----------------------------------------|-------------------------|-----------------------------------|----------------------|---------|
| Non-poly (model 1) | 3.00                        | 0.54                                    | 0.81                    | 722.89                            | 1 vs. 2              | < 0.001 |
| Quadratic (model 2) | 2.99                        | 0.63                                    | 0.67                    | 634.63                            | 2 vs. 3              | < 0.001 |
| Cubic (model 3)   | 3.00                        | 0.65                                    | 0.63                    | 611.59                            | 3 vs. 4              | < 0.001 |
| Quartic (model 4) | 3.20                        | 0.67                                    | 0.61                    | 592.7                             | 4 vs. 5              | 0.020   |
| Quintic (model 5) | 2.89                        | 0.68                                    | 0.60                    | 588.58                            |                      |         |

Model 4 had the lowest AIC and within variability (SD) but high ICC values, the key selection criteria for a reliable model, and hence it was used to model *M. tuberculosis* killing rates.
| Variable                  | All       | RHZE (n = 7) | Injectable ± BDQ (n = 21) | ± All-oral BDQ (n = 9) | p-value |
|---------------------------|-----------|--------------|---------------------------|-----------------------|---------|
| Median age (IQR)          | 37 (32 – 49) | 30 (29 – 33) | 42 (34-54)                | 36 (33- 44)          | 0.038   |
| Male (%)                  | 27 (73)   | 4 (57)       | 18 (86)                   | 5 (56)                | 0.125   |
| Chest cavity, n (%)       | 29 (78)   | 7 (100)      | 14 (67)                   | 8 (89)                | 0.163   |
| Probable TB, n (%)        | 34 (92)   | 7 (100)      | 18 (86)                   | 9 (100)               | 0.568   |
| HIV positive, n (%)       | 11 (20)   | 0 (0)        | 3 (14)                    | 8 (89)                | 0.001   |
| TB/Silicosis, n (%)       | 7 (19)    | 1 (14)       | 4 (19)                    | 2 (22)                | 0.731   |
| Malnourished, n (%)       | 22 (59)   | 4 (57)       | 11 (52)                   | 7 (78)                | 0.432   |
| Retreatment, n (%)        | 23 (62)   | 5 (71)       | 14 (67)                   | 4 (44)                | 0.528   |
| Median BMI (IQR)          | 18 (15 – 19) | 17 (15 – 20) | 18 (16 – 20)              | 17 (15 – 18)         | 0.301   |
| Median days spent before care (IQR) | 84 (60 – 196) | 85 (68 – 93) | 84 (56 – 196)              | 88 (68 – 365)       | 0.778   |

BDQ, bedaquiline; BMI, body-mass-index; injectable± BDQ, kanamycin with or without BDQ and IQR, interquartile range. Probable TB was defined as the presence of radiological changes including cavity, infiltrates, nodules, hilar lymphadenopathy and aortopulmonary window adenopathy on chest radiograph. P value was computed to compare RHZE, injectable± BDQ, kanamycin with or without BDQ regimens.
Table 3. Mean daily *M. tuberculosis* killing rates (log10 eCFU/mL) and corresponding burden at day 0 (baseline) and 112 of treatment

| Treatment regimens | Mean *M. tuberculosis* killing rates | Mean (95% CI) *M. tuberculosis* load |
|--------------------|-------------------------------------|-------------------------------------|
|                    | Unadjusted model for covariates | Adjusted model for covariates | Day 0 (baseline) | Day 112 * |
| 1. Reference (injectable-BDQ free) | -0.18 (-0.27 to -0.08) | -0.17 (-0.23 to -0.12) | 4.73 (4.13 – 5.32) | 2.77 (2.51- 3.04) |
| 2. Injectable-bedaquiline | -0.48 (-1.25 to +0.28) | 0.239 | -0.62 (-1.05 to -0.20) | 0.019 | 4.63 (3.95 – 5.47) | 2.08 (1.81 - 2.36) |
| 3. All-oral bedaquiline | -0.26 (-0.48 to +1.00) | 0.507 | -0.35 (-0.65 to -0.13) | 0.054 | 5.36 (4.65 – 6.08) | 2.47 (2.20 - 2.74) |
| 4. Standard RHZE | -0.23 (-0.57 to +1.02) | 0.593 | -0.29 (-0.78 to +0.22) | 0.332 | 5.17 (4.36 – 5.99) | 2.51 (2.18 - 2.85) |

†Baseline mean *M. tuberculosis* load in all regimens were comparable (ANOVA, p = 0.453). An asterisk (*) denotes p-values for mean difference in *M. tuberculosis* load for regimen pairwise comparison at day 112: regimen 1 & 2, p < 0.001; regimen 2 & 3, p = 0.031; regimen 1 & 3, p = 0.077; and regimen 2 & 4, p = 0.040. Reference regimen was the injectable-bedaquiline (BDQ) free regimen composed of kanamycin (KAN), levofloxacin (LFX), pyrazinamide (PZA), ethionamide (ETH) and Cycloserine (CS); Injectable-bedaquiline regimen was comprised of KAN, BDQ, LFX, PZA and ETH; All-oral bedaquiline regimen contained BDQ, LFX, linezolid (LZD), PZA and ETH; and the RHZE for rifampicin, isoniazid, PZA and ethambutol (E). Covariates adjusted included baseline bacterial load, cavity, gender, HIV and silicosis, *M. tuberculosis* killing rates varied among regimens.
Table 4. Hazard ratio (HR) of *M. tuberculosis* killing in Cox Proportion-Hazard model

| Predictor Variable | Unadjusted model | Adjusted model |
|--------------------|------------------|----------------|
|                    | HR (95% CI)      | p-value        | HR (95% CI) | p-value |
| Male gender        | 0.86 (0.40 – 1.85) | 0.705          | 2.44 (0.82 – 7.24) | 0.109  |
| TB/Silicosis       | 0.20 (0.10- 0.88) | 0.028          | 0.12 (0.03 – 0.49) | 0.003  |
| TB/HIV             | 2.26 (1.07 -4.77) | 0.033          | 0.88 (0.31 – 2.50) | 0.813  |
| Cavitary disease   | 0.38 (0.17 - 0.86) | 0.021          | 0.85 (0.17 – 2.70) | 0.790  |
| Positive chest x-ray | 0.57 (0.17 – 1.88) | 0.354          | 0.23 (0.03 – 1.62) | 0.790  |
| High *Mt* load     | 0.72 (0.54 -0.97) | 0.033          | 0.26 (0.13 – 0.54) | < 0.001|
| Retreatment        | 1.02 (0.51 - 2.05) | 0.958          | 0.59 (0.24 – 1.44) | 0.248  |
| All-oral bedaquiline | 1.58 (0.61 - 4.04) | 0.344          | 12.37 (2.87 – 53.30) | 0.001  |
| Injectable- bedaquiline | 4.63 (1.64 – 13.09) | 0.004          | 14.31 (3.49 – 58.65) | < 0.001|
| Standard RHZE      | 1.43 (0.53 – 3.89) | 0.482          | 3.25 (0.90 – 11.73) | 0.072  |
| High initial *Mt* killing rate | 5.96 (2.03 – 17.48) | 0.009          | 4.81 (1.39 – 16.65) | 0.013  |

All-oral bedaquiline regimen was comprised of Bedaquiline (BDQ), levofloxacin (LFX), linezolid (LZD), pyrazinamide (PZA) and ethionamide (ETH). Injectable-bedaquiline is a modified regimen comprised of kanamycin (KAN), BDQ, LFX, PZA and ETH. Standard RHZE included rifampicin (H), isoniazid (H), PZA and ethambutol (E).
59 (472 sputa) consented

22 excluded
- 4 died
- 7 lost to follow up
- 11 negative baseline
  TB-MBLA & LJ culture

37 (296 sputa) analyzed

30 (240 sputa) RR/MDR-TB
7 (56 sputa) DS-TB

Treatment regimens

- All-oral BDQ (n = 8)
- Injectable-BDQ (n = 9)
- Injectable-BDQ free (n = 13)
- Standard RHZE (n = 7)

Figure 1.
Figure 2.
Figure 3. Kaplan-Meier Curves for TB-MBLA conversion per regimen

log-rank, p < 0.001

Treatment regimens
- Injectable-bedaquiline free (n = 13)
- All-oral bedaquiline (n = 9)
- Injectable-bedaquiline (n = 8)
- Standard RHZE (n = 7)

Probability of sputum conversion

Treatment duration in days
Figure 4.