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Safety of high-dose amikacin in the first week of all-oral rifampicin-resistant tuberculosis treatment for the prevention of acquired resistance (STAKE): protocol for a single-arm clinical trial

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ABSTRACT

Introduction An effective rifampicin-resistant tuberculosis (RR-TB) treatment regimen should include prevention of resistance amplification. While bedaquiline (BDQ) has been recommended in all-oral RR-TB treatment regimen since 2019, resistance is rising at alarming rates. This may be due to BDQ's delayed bactericidal effect, which increases the risk of selecting for resistance to fluoroquinolones and/or BDQ in the first week of treatment when the bacterial load is highest. We aim to strengthen the first week of treatment with the injectable drug amikacin (AMK). To limit the ototoxicity risk while maximising the bactericidal effect, we will evaluate the safety of adding a 30 mg/kg AMK injection on the first and fourth day of treatment.

Methods and analysis We will conduct a single-arm clinical trial on 20 RR-TB patients nested within an operational study called ShoRRT (All oral Shorter Treatment Regimen for Drug resistant Tuberculosis). In addition to all-oral RR-TB treatment, patients will receive two doses of AMK. The primary safety endpoint is any grade 3–4 adverse event during the first 2 weeks of treatment related to the use of AMK. With a sample size of 20 patients, we will have at least 80% statistical power to support the alternative hypothesis, indicating that less than 14% of patients treated with AMK experience a grade 3–4 adverse event related to its use. Safety data obtained from this study will inform a larger multicountry study on using two high doses of AMK to prevent acquired resistance.

Ethics and dissemination Approval was obtained from the ethics committee of Rwanda, Rwanda Food and Drug Authority, Universitair Ziekenhuis, the Institute of Tropical Medicine ethics review board. All participants will provide informed consent. Study results will be disseminated through peer-reviewed journals and conferences.

Trial registration number NCT05555303.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ In this trial, every single rifampicin-resistant tuberculosis (RR-TB) patient in Rwanda will be evaluated for enrolment, with results generalisable to the Rwandan setting.
⇒ This pragmatic trial relies on routine data collection and monitoring procedures in the context of a strong RR-TB control programme in Rwanda.
⇒ This study is not powered to study the efficacy of the modified regimen in preventing drug resistance.
⇒ In this trial, proxy endpoints known to correlate with treatment response, such as the bactericidal effect of the regimen, will be analysed.

INTRODUCTION

Rifampicin-resistant tuberculosis (RR-TB) is unresponsive to rifampicin, the most potent anti-TB drug. Nowadays, RR-TB is mostly driven by transmission between individuals. When RR-TB regimens are not robust, with poor resistance prevention activity, extensively drug-resistant TB (XDR-TB) can develop, which is RR-TB also resistant to isoniazid, fluoroquinolones (FQs) and additionally to bedaquiline (BDQ) or linezolid, compromising remaining treatment options.[1] In Rwanda, in July 2014, and following high success rates in multiple countries,[2 3] the injectable-containing shorter treatment regimen (Inj-STR; 9–11 months of treatment including 4 months of a second-line injectable drug (SLI)) replaced the long 20-month RR-TB regimen.[4] In July 2021, following

updated WHO guidelines, the new all-oral STR was introduced.[1] This 9-month all-oral regimen comprises BDQ (used for 6 months), in combination with FQ (levofloxacin/moxifloxacin), ethionamide, ethambutol, isoniazid (high dose), pyrazinamide and clofazimine (for 4 months, with the possibility of extending to 6 months if the patient remains sputum smear positive at the end of 4 months); followed by treatment with FQ, clofazimine, ethambutol and pyrazinamide (for 5 months). This all-oral STR was introduced in the context of operational research (ShORRT: All-oral shorter treatment regimen for multidrug and RR-TB (MDR/RR-TB): Evaluating its effectiveness, safety and impact on the quality of life of patients in Rwanda).[1] Regarding the prevalence of resistance in Rwanda, in a study by Habimana-Mucyo *et al* the prevalence of RR-TB among all bacteriologically confirmed pulmonary TB patients from 2019 2020 with available DST results was 1.4% for new TB cases and 4.9% for previously treated cases. Second-line DST results were available for 48 (65.8%) of RR-TB cases. No resistance to FQ was observed, while only one patient exhibited RR-TB with resistance to all second-line injectable agents.

Recent studies have shown that all-oral MDR/RR-TB regimens, including BDQ and an FQ, but without SLIs, are associated with acquired resistance.[5] Specifically, these studies report a bacteriologically adverse outcome with acquired BDQ resistance of around 2.3%,[6] which is much higher than the rates of acquired rifampicin resistance with the first-line regimen (0.1%). Other reports from Pakistan[7] and Moldova[8] showed that 6 (20%) of 30 and 4 (15%) of 26 patients had acquired BDQ resistance under BDQ containing regimens, respectively, far exceeding rates in clinical trials. Adherence may also contribute to acquired resistance, although its effect is difficult to evaluate in programmatic settings where it is seldom reported. In our trial and for all patients included in the ShORRT master study, adherence will be reported.

The relatively high frequency of acquired BDQ resistance may be due to its delayed killing activity, which reaches its peak only after 1 week of treatment.[9] The slow penetration of BDQ inside the granuloma may contribute to this delayed onset of action.[10] Also, BDQ has been shown to trigger bacteriostasis, enabling transient bacterial survival.[11] During that time, initially, FQ-resistant mutants continue to multiply, resulting in an estimated 2 log concentration of FQ-resistant bacilli for BDQ to kill once its action starts. As minority populations of initially FQ-resistant bacilli may remain undetected by rapid molecular tests such as Xpert XDR (Cepheid, USA)[12] a baseline FQ-susceptible result is not fully reliable.

During this crucial first week of treatment, when the bacillary load and multiplication are highest, a weak regimen can select resistance-conferring mutants. To address this weakness, a potential solution is to strengthen the first week of the all-oral STR with a powerful bactericidal drug, such as SLIs.[3] A study[3] analysing the effect of using 2 months of kanamycin instead of the standard 4(+) months on recurrence and acquired FQ resistance in patients treated with a gatifloxacin-based STR in Bangladesh showed that 2 months of kanamycin was insufficient to prevent recurrence with acquired resistance to gatifloxacin, indicating that injectable mediated resistance prevention is important to prevent acquired resistance. In another study led by CDC, Atlanta[13 14] has shown that the short regimen including SLI activity of SLIs had a strong effect on the prevention of FQ-resistance acquisition compared with regimens not approved by the Green Light Committee. This study[13] also showed that baseline resistance to the SLIDs had the greatest impact on the risk of acquired XDR TB (before 2020, the definition of XDR was resistance to rifampicin, isoniazid, any FQ and any SLID). With baseline resistance limited to first-line drugs, the risk of acquired XDR was 2.4%. With baseline resistance to an FQ, the risk of acquired XDR was 16.7%. With baseline SLIDs resistance, the risk of acquired XDR was 36.8%–46.0%, depending on the specific drug. Together with their pharmacokinetic and pharmacodynamic characteristics, this makes them a good candidate to strengthen the first week of all-oral STR. Notably, in Pakistan, where BDQ was used either with or without SLIDs acquired BDQ resistance was significantly more frequent when SLIDs did not protect BDQ (53.8% (7/13) vs 7.7% (1/13); OR 9.6; 95% CI 1.3 to 70.5) and in patients previously treated with a SLID-containing second-line regimen (58.3% (7/12) vs 7.1% (1/14); OR 12.3; 95% CI 1.6 to 92.0).[6] Importantly, the STREAM stage 2 trial, which served as phase 3 trial of BDQ, included a 6-month arm with 2 months of kanamycin with excellent outcomes and no acquired BDQ resistance.

To constitute a strengthened regimen, we will add amikacin (AMK), the most potent SLI,[15] to the currently used all-oral STR in the first week of treatment, in two doses of 30 mg/kg; a first dose on day one and a second dose on day 4.

One of the reasons why WHO recommended replacing the SLI by BDQ in 2021 was the concern about SLIs associated ototoxicity. The strategy to be evaluated, with two high doses of AMK in the first treatment week, is expected to be safe for several reasons. First, toxicity is correlated with the lifetime cumulative dose of any SLI.[16] Administration of 2 doses of 30 mg/kg AMK is only a small fraction (1.6%–3.3%) of the cumulative exposure from daily 10–15 mg/kg doses during either 4 or 8 months, used for many years as standard RR-TB treatment.[17] Second, a previous study on 25 mg/kg intermittent use showed adverse events (AE) starting from a cumulative dose of 150 mg/kg[18] while we achieved a cumulative dose of 60 mg/kg. Also, TB bacilli show a very long lag phase after a single exposure to an injectable drug, the 'postantibiotic effect', justifying their use every few days, with intermittent (eg, thrice weekly) dosing being as effective as daily in clinical trials.[18 19] The choice for two doses of 30 mg/kg of AMK is informed by the efficacy of AMK, which is correlated with the peak serum concentration (or Cmax) over minimum inhibitory concentration (MIC).[16] A hollow-fibre model study showed that AMK's

bactericidal effect was the highest when the Cmax/MIC ratio was at least 10 at the site of infection.[16] Considering poor penetration of SLIs in lung tissue, this translates into a serum Cmax/MIC ratio of 75.[16] With two doses of 30 mg/kg, we aim to obtain the highest efficacy without causing harm.

Given the difficulty of enrolling large numbers of RR-TB patients, the very long turnaround time between designing randomised clinical trials (RCTs) and having the results published, the many different drug combinations to be tested, and the urgent need to improve treatment regimens protecting against acquiring resistance, adaptive trial designs need to be explored. We, therefore, designed a single-arm trial with a fixed safety threshold (see justification in the Methods section). We hypothesise that not a single patient of 20 patients enrolled will experience a grade 3 or 4 AE, thus with the upper bound of the 95% CI below 14%. We aim for future RR-TB patients to benefit from the use of AMK without causing ototoxicity. Safety data obtained from this study will inform a subsequent larger multicountry study on the efficacy and acceptability of two doses of AMK to prevent acquired BDQ resistance in patients treated with all-oral regimens.

Objectives and endpoints

Primary and secondary objectives

Primary objective

Assess whether less than 14% of patients treated with the AMK-strengthened all-oral STR will experience a grade 3–4 AE likely or definitely related to the use of AMK after 2 weeks (±3 days) of treatment.

Secondary objectives

- Describe (type, grading) the occurrence of AEs that are considered as likely or definitely related to the use of AMK, at the end of treatment week 2 (±3 days).
- Describe the AMK cumulative exposure stratified by AE occurrence.
- Describe postinjection pain on a Visual Analogue 0–10 Pain Scale (The Wong-Baker Faces Pain Rating Scale) at 0, 15 min, 30 min and 60 min after the injection of AMK with lidocaine on days 1 and 4, as well as the next morning.[20]
- Describe all AEs, by their grade and their relationship with anti-TB drugs, for the entire treatment duration.
- Describe treatment and post-treatment outcomes, at the end of treatment and after post-treatment follow-up. The follow-up includes ambulatory visits at 6 and 12 months after treatment end. Treatment outcomes are shown in the master ShORRT protocol, are the same as those defined by WHO and are reported in online supplemental file.[21]

Primary and secondary endpoints

Primary endpoint as a measure of safety

Any grade 3–4 AE during the first 2 weeks of treatment, assessed as likely or definitely related to the use of AMK.

Secondary safety endpoints

- Ototoxicity on audiometry, by grade, assessed as likely or definitely related to the use of AMK, measured at the end of week 2.
- Nephrotoxicity, by grade, assessed as likely or definitely related to the use of AMK, measured at the end of treatment week 2.
- Any severe adverse event (SAE), assessed as likely or definitely related to the use of AMK, measured at the end of treatment week 2.
- Any other AE, by grade, assessed as likely or definitely related to the use of AMK, measured at the end of treatment week 2.
- Postinjection pain on a 0–10 pain scale (The Wong-Baker Faces Pain Rating Scale) at 0, 15 min, 30 min and 60 min after the injection of AMK, with lidocaine, as well as the next morning.
- Any AE, by grade and relationship with TB drugs, for the entire treatment duration.

Secondary treatment response endpoints

- Colony-forming units (CFUs) counts on semiquantitative culture on six-well thin-layer agar plates (serial dilutions of inoculum) and time to culture positivity on liquid culture (Mycobacteria Growth Indicator Tube (MGIT), Becton Dickinson, USA), tests that are used to assess reduction in bacillary burden in studies assessing the early bactericidal activity of regimens.[22]
- Month of stable (without reversion) culture conversion.
- End-of-treatment outcomes (see online supplemental file).
- Treatment outcomes at 12 months post-treatment (end-of-treatment outcome corrected for early relapse, see online supplemental file with outcomes definitions).
- Acquired resistance to BDQ, FQs, AMK through target deep sequencing on paired baseline and failure sputa.

METHODS AND ANALYSIS

Study design and sample size

This is a single-arm clinical trial conducted according to protocol V.5.0 approved on 31 January 2023. Patients will be coenrolled in the ShORRT operational research study. All patients consecutively diagnosed with RR-TB in Rwanda will be assessed for eligibility and 20 patients will be enrolled in STAKE. Based on current trends, we expect about 6–12 months to enrol 20 patients.

Study setting

This study will be conducted at the Kabutare hospital, which is a dedicated RR-TB treatment centre located in the southern province of Rwanda. During admission, independently from the participation to this study, patients receive free TB treatment, nutrition and psychosocial support while being clinically monitored. Once clinically stable with at least one negative culture, patients are discharged for ambulatory treatment, with

Table 1 Dosage of amikacin and lidocaine by weight band

Weight band	Amikacin in mL (250 mg/mL in 2 mL vials)	Amikacin in mg	Range amikacin, mg/kg	Lidocain in ml (20 mg/mL)	Lidocain in mg	Range lidocain mg/kg
23–30	3	750	32.6|25.0	0.4	8	0.35|0.32
31–38	4	1000	32.3|26.3	0.5	10	0.32|0.38
39–45	5	1250	32.1|27.8	0.5	10	0.26|0.36
46–54	6	1500	32.6|27.8	0.5	10	0.22|0.36
55–64	7	1750	31.8|27.3	0.5	10	0.18|0.37
65–70	8	2000	30.8|28.6	0.5	10	0.15|0.35
71–78	9	2250	31.7|28.8	0.5	10	0.14|0.35
79–87	10	2500	31.6|28.7	0.5	10	0.13|0.35
88–95	11	2750	31.3|28.9	0.5	10	0.11|0.35
>96	12	3000	31.3|30.0	0.5	10	0.10|0.33

directly observed therapy at a health facility near their home.

Inclusion and exclusion criteria

Inclusion criteria

- Has bacteriologically or molecularly confirmed TB with evidence of resistance to at least rifampicin (ShoRRT).
- Patient having provided written informed consent.
- Age >18 years and <65 years old.

Exclusion criteria

- Any audiometry abnormality (grade 1 or higher) on baseline audiometry, using the Average Hearing Loss at frequencies 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz.
- History of kidney disease or baseline estimated glomerular filtration rate below or equal to 60 mL/min/1.73 m^2.
- Pregnant or breastfeeding women.
- History of previous injectable-based TB treatment.
- Resistance to second-line injectables.
- Patient on non-steroidal anti-inflammatory drugs (NSAID) or on diuretics.

Intervention description

AMK will be available in 2 mL vials (500 mg/2 mL solution) and will be administered on day 1 and day 4 according to the weight bands (table 1) if baseline audiometry is normal. Lidocaine solution (1 mL of 2% lidocaine solution) will be admixed in the same AMK syringe by the study nurse to reduce injection-site pain.[20 23] Intramuscular injections will be administered with a 21 gauge 1.5 inch needle in the dorsogluteal area, according to standard local practice. Patients who received a first administration with 30 mg/kg AMK on day 1, and with creatinine clearance below or equal to 60 mL/min before the second administration or show an increase in hearing loss greater than 20 dB at any frequency relative to baseline values will not receive a second administration of AMK on day 4. A schedule of assessments is provided in table 2. Post-AMK injection pain will be evaluated via the Wong-Baker Faces Pain Rating Scale.[20]

Laboratory procedures

On admission, patients provide sputum for confirmation of RR by Xpert Ultra, and exclusion of SLI resistance by Xpert XDR, at Kabutare hospital. Once the patient is found to meet inclusion criteria, the informed consent is obtained before starting RR-TB treatment. Patients will be requested to provide three sputa collected at different time points including an overnight sputum as described in previous studies.[24] For the treatment monitoring, two overnight sputa will be collected. The overnight sputum collected at baseline will be aliquoted including one with ethanol. The sputa will be used for diverse microbiological testing including quantified smear microscopy, mycobacterial culture in MGIT with time to positivity recorded and CFU counts. All sputa are shipped to the National Reference Lab (NRL) in Kigali for testing. Results of microbiological tests performed in routine practice such as cultures and baseline drug-susceptibility testing will also inform this study. Besides sputa, blood samples are collected to measure AMK levels in serum at the University Medical Center Groningen, Groningen (UMCG), the Netherlands. We will measure serum levels 2 hours and 6 hours after the injection and before the second dose. This will allow us to back-calculate the Cmax but also estimate the 24 hours area under the time-concentration curve.

Laboratory analysis

Xpert MTB/RIF ultra and MTB/XDR

Before recruitment into the study, RR-TB is confirmed with ultra and SLIs resistance is excluded by XDR.

Routine microscopy and culture on solid and liquid media

Standard procedures are followed. Sputum will be processed within 72 hours and sputum specimens will be decontaminated using N-Acetyl-L-Cysteine Sodium hydroxide followed by neutralisation with phosphate buffer, centrifuged and the pellets (0.5 mL) inoculated

Table 2 Schedule of assessments

	Day 0	Day 1	Day 3* or 4	Day 7±1 day	Day 14±3 days	Month 1/2†	Months 3–9†	PT 6/PT 12†
Informed consent	X							
Pregnancy test	X							
Xpert XDR	X							
Ethanol sputum sample for eventual a posteriori analysis	X					X	X	X
AMK intramuscular injection (30 mg/kg)		X	X					
Pain scale‡		X	X					
Quantified sputum smear microscopy	X	X	X	X	X	X	X	X
Solid and liquid culture§	X	X	X	X	X	X	X	X
Audiometry¶	X		X		X	X		
Blood sampling**		X	X					
Creatinine clearance	X		X		X			
Adverse events form††					X	X	X	X

*Audiometry and creatinine clearance (+AMK trough level) will be determined on day 3 to allow time for physician interpretation and clearance before D4 AMK injection.
†Plus/minus 7 days.
‡Postinjection pain on a 0-10 pain scale (The Wong-Baker Faces Pain Rating Scale) at 0, 15 min, 30 min and 60 min after the injection of AMK with lidocaine, as well as the next morning.
§Culture: Time to positive culture and number of culture forming units on days 1 (first day of RR-TB treatment, sample collected before the first dose), 4 (sample collected just before the second dose), 7 and 14. For monthly culture, as per routine monitoring, only data on positivity (positive, negative, contaminated, not done) will be collected.
¶Audiometry: At baseline (day 0, before start of treatment), day 3 (before second administration of AMK), day 14, month 1 (±7 days) and month 2 (±7 days) (as ototoxicity may emerge with delay since administration) and any time thereafter in case of hearing disturbances.
**The concentrations of AMK: just before administration of AMK (concentration of drugs other than AMK) and 2 and 6 hours after the administration of AMK when the first (day 1) and second dose (day 4) of AMK are administered.
††The table only included the schedule of cumulative adverse events report forms. However, throughout directly observed therapy, patients will be monitored for any potential adverse event actively and by passive reporting.
AMK, amikacin; PT, past-treatment month; TLA, thin layer agar; XDR, extensively drug-resistant.

in 2 Löwenstein-Jensen (LJ) tubes and one MGIT tube. For LJ, incubation will be 8 weeks with a weekly reading before declaring negative, while in MGIT, incubation will be in the automated BACTEC-MGIT960 for 42 days. The time to positivity in MGIT and colony counts on LJ will be registered. Positive MGIT tubes will be screened for purity using a blood agar plate.

The smears prepared from the leftover homogenised sputum sediment will be stained using auramine and fluorescein diacetate (FDA) vital staining for LED-FM examination and grading (scanty, 1+, 2+, 3+).

CFU counts

Leftover homogenised sputum sediment will be used to prepare a range of 10-fold dilutions from 10^{-2} to 10^{-5}. From each dilution, 100 µl will be plated in quadruplicate on 7H11 agar plates. The CFUs will be counted at the dilution that yielded 20–200 visible colonies after 4 weeks of incubation at 37°C.

Whole genome sequencing and Deeplex-MycTB targeted deep sequencing to determine a comprehensive initial resistance profile

In an EDCTP-funded project called DIAMA, the NRL installed an Illumina MiniSeq device to validate the Deeplex-MycTB assay.[25] All baseline sputa and all failure and relapse sputa and isolates will be preserved in ethanol to determine resistance patterns using Deeplex and whole genome sequencing allowing to distinguish true failures and relapses from reinfections and showing acquired resistance, if present.

Table 3 Audiometry and grading of otoxicity

	Hearing loss compared with baseline*
Normal values	0–20 dB
Grade 1: light	21–40 dB
Grade 2: moderate	41–70 dB
Grade 3: severe	71–90 dB
Grade 4: deafness	>90 dB

*Frequencies between 500 Hz and 4000 Hz are those of normal conversation. The higher frequencies (4000–8000 Hz) are affected first; the frequencies of the human voice are reached in a second step. Hearing loss becomes noticeable to patients at frequencies <4000 Hz if it reaches 25–30 dB. The grading table is adapted from the National Agency for Research on AIDS and Viral Hepatitis (ANRS) 2008 scale for rating the severity of adverse events in adults. For the hearing screening we use a portable audiometer (Interacoustics AS608). If any abnormality is reported, the treatment is stopped and the patient is referred to an ENT specialist.
ENT, ear-nose-throat.

Table 4 Renal toxicity according to creatinine clearance levels

	Nephrotoxicity (mL/min/1.73 m^2)
Normal values	>90
Grade 1: light	60–89
Grade 2: moderate	59–30
Grade 3: severe	15–29
Grade 4: renal failure	<15

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) staging for estimated glomerular filtration rate. Creatinine is collected at baseline, days 3–4 before the second AMK injection, day 14 and then monthly using the CKD-EPI 2021 formula. If the creatinine clearance is inferior to 60 mL/min after the first AMK injection, the second injection is not administered.
AMK, amikacin.

AMK serum levels

Two and 6 hours after the administration of AMK on days 1 and 4, and just before administration of AMK on day 4, venous blood will be drawn. The preinjection trough level of AMK is drawn on day 3, together with determination of the creatinine level. After collection, blood samples will be centrifuged, serum pipetted and frozen (at −20°C) before shipment to ensure stability. All samples will be shipped on dry ice to the UMCG for bioanalysis and determination of the AMK concentration based on turbidimetry using a validated immune-assay technique on an Architect C8000 (Abbott).

Safety assessment and reporting

RR-TB patients will remain hospitalised at the RR-TB clinic until they have achieved clinical stability and have tested negative in one culture.

Any hearing loss >20 dB from baseline at any frequency will be considered an AE. The audiogram will be repeated the next day and an ear-nose-throat specialist assesses any patient with a confirmed abnormal audiogram. Throughout directly observed therapy, patients will be monitored for any potential AE per the schedule of events (table 2) and by passive reporting. If any AE occurs, it will be promptly recorded, graded according to established tables 3 and 4 for nephrotoxicity and ototoxicity and to the Common Terminology Criteria for Adverse Events for other adverse events and treated. If the event is graded 4 or meets other criteria for serious AE (such as hospitalisation), an 'SAE form' will be completed and promptly submitted to the principal investigator (PI) and relevant pharmacovigilance authority at the latest by the next working day. Line listings of eventually reported SAEs will be sent quarterly to the Data and Safety Monitoring Board which includes a nephrologist, a pneumologist, a statistician and two RR-TB experts. They will also be sent on a 6-monthly basis to the PI and study coordinator in Rwanda who will submit these to the respective authorities Food and Drugs Authority, and/or ethical commission (EC) in Rwanda, and on a yearly basis to the Institutional Review Board (IRB) of the Institute of Tropical Medicine (ITM) and the EC of the Universitair Ziekenhuis Antwerpen (UZA).

Data management and monitoring

Data from these data sources will be encoded in an electronic database (REDCAP) by the local research team. Data entry will be performed by the local research team, supervised by the PI. The ITM Clinical Trial Unit (CTU) will monitor data quality. Roles and responsibilities are described in detail in the data management plan, including details on the quality and validation of the systems used as well as a description of the data cleaning process. Data sources are paper treatment cards and treatment and laboratory registers.

In accordance with Findable, Accessible, Interoperable, Reusable (FAIR) principles and the General Data Protection Regulation (GDPR), participant-level data might be shared in an anonymised manner to allow secondary research after completion of the study and after formal review and approval of such secondary research.

Regular data review and data monitoring and cleaning for quality control are performed quarterly in accordance with Good Clinical Practice guidance requirements by the PI, the CTU at ITM and national RR-TB focal point. In case the procedures would have to change, an amendment will be developed and submitted to the relevant ethics review bodies. All analyses of clinical trial samples will be carried out in compliance with Good Clinical Laboratory Practice.

Statistical methods for primary and secondary outcomes

A review showed that 10.2% (95% CI 6.3% to 16.0%) of patients interrupted AMK when used for at least 4 months.[26] Since this is probably an underestimation, as some grade 3 AE may have been overlooked and

other studies report far more severe ototoxicity than 10%,[6] we used a threshold of 14%.

For the primary analysis, we will calculate the rate and the one-sided 95% CI around the proportion with the primary safety endpoint and assess whether the upper bound of the CI is lower than 14%. Other statistics will be descriptive.

With 20 patients we will be able to assess the primary safety endpoint, the proportion of patients with a grade 3–4 AE likely or definitely related to the use of AMK.

- Null hypothesis: 14% of patients treated with AMK have a grade 3–4 AE likely or definitive related to the use of AMK.
- Alternative hypothesis: less than 14% of patients treated with AMK have a grade 3–4 AE likely or definitely related to the use of AMK.
- P value for a binominal test with p0=0.14 (p=0.14 under the null hypothesis) for 0 events is $(1-0.14)^{20} = 0.049 < 5\%$.
- In case the probability of having a grade 3–4 AE likely or definitive related to the use of AMK would be 1%, then the chance for having 0 of 20 patients with this safety endpoint is 81.8% ($(1-0.01)^{20} = 0.818 = 81.8\%$). Thus, we have a bit more than 80% power, or more if the probability of having this safety endpoint is less than 1%.

With 20 patients, we have at least 80% power to reject the null hypothesis of p0=0.14 in favour of the alternative of p<0.14 assuming that p≤0.01. To describe turnaround times and testing coverage no formal sample size calculation is needed. For the assessment of treatment response endpoints, we did not provide a sample size calculation. Sample size calculations for treatment response endpoints, including acquired resistance, will be developed for a larger multicountry study, building on data provided by the present study.

Confidentiality

The study will be performed in compliance with the European Union's GDPR. The study database and any other paper documents or electronic files used for data management and analysis will only include pseudonymised data. Participants will be identified by their routine ID number as well as a unique number assigned for each TB episode. The register correlating patient ID with the patient's demographic data is kept in a secured lock in the clinical staff office.

Ethics and dissemination

Approval was obtained from the ethics committee of Rwanda (N459/RNEC/2022) and Rwanda Food and Drug Authority (DIS/FMT/050), Universitair Ziekenhuis Antwerp (No 3330—Edge n/a—BUN B3002022000108 of 08/08/22) and the ITM ethics review board (No1567/22 of 14/06/22). Written informed consent to participate will be obtained from all participants.

Findings will be shared through an abstract presentation at scientific conferences and publication in peer-reviewed journals in accordance with the Consolidated Standards of Reporting Trials statement. Coauthors will include staff from the Rwandan Biomedical Centre, the RR-TB clinics and collaborators.

DISCUSSION

New drugs for RR-TB have allowed to shorten treatment to 6 months,[27 28] yet subsequent treatment options for patients who experience treatment failure of relapse remain limited. Worryingly, resistance to BDQ increases fast after its widespread implementation in current all-oral regimens.[10] Nevertheless, the prevention of acquired drug resistance is not prioritised during real-world implementation. Acquired resistance is often a secondary endpoint for which studies are not powered to provide meaningful results.[29 30] By adding two injections of high-dose AMK to a BDQ-containing regimen, we aim to prevent acquired resistance to the core drugs while avoiding SAEs. First, we will test safety in a small cohort of 20 patients.

While RCTs are considered the reference standard for evaluating the safety and efficacy of interventions, their implementation in the field of RR-TB poses significant challenges. First, there is the relatively low number of patients diagnosed with RR-TB, and the lengthy enrolment, treatment and follow-up periods. Second, the standard clinical development process is difficult to apply in RR-TB. While the traditional scientific learning process follows an iterative cycle of exploration and confirmation, clinical development often starts with the definition of a specific regimen and works backward to identify the necessary data to justify the adoption of that regimen.[31] Third, such time-consuming and expensive phase 3 RCTs may yet fail to inform policy decisions[32] when pilot trials have not adequately explored crucial factors.[32] Indeed, in the field of RR-TB, identifying the perfect regimen a priori is particularly difficult due to the vast number of drug combinations to explore, the high rate of adverse events due to drug toxicity and the long follow-up time to ascertain treatment outcome.[33] The consequential delays in obtaining results can render trial findings on studied regimens outdated by the time of publication.[34]

To address these challenges, we use a stepwise approach. A multistage multiarm adaptive design would be ideal, if the cohort would be large enough to enrol on multiple arms at the same time, selecting most performant regimens over time.[35] However, due to the constraint of a limited maximum number of patients eligible for enrolment on a yearly basis, we will subsequently use small studies. Each study is a stepping stone towards conducting a larger comparative trial. Conducting tests and acquiring knowledge about the intervention in smaller cohorts increases the chances of identifying a valid and evidence-based hypothesis, ultimately mitigating the risk of inconclusive larger RCTs. These individual studies should be

both confirmatory, ensuring adequate statistical power for primary analysis and decision-making, and exploratory, with features that aid in the critical learning process.[31 32] Moreover, they should include evaluation of minimal clinically important differences, that is, the smallest change in outcome that the patients would consider important. Following this reasoning, the present study was designed to have sufficient statistical power to address the primary safety endpoint. Additionally, the study included exploratory effectiveness features which can offer valuable insights to guide future interventions. Also, a planned substudy will investigate patient-reported outcomes. Finally, although a control group is not formally included, our study is part of the larger ShORRT study, which assesses the effectiveness of the background 9-month, all-oral regimen with BDQ. This setup enables comparisons of treatment response outcomes between regimens with and without AMK, which will be investigated in another planned substudy.

Our trial has important strengths. Since every single RR-TB patient in Rwanda will be evaluated for enrolment in this study, we expect the findings to be generalisable to the Rwandan setting. Also, the design used is feasible for routine RR-TB care, using routine data collection, analysis and monitoring procedures which informed numerous previous publications.[24 36 37] Therefore, we expect prospectively collected data to be reliable and complete.

The study design also has some weaknesses. By adopting a step-by-step approach to testing new interventions for RR-TB, there will be an increase in the number of phase 2 studies conducted. While this approach is expected to improve the quality of phase 3 trials, it may also extend the time required to introduce an effective new treatment. Also, to provide adequate power, we chose a fixed threshold that was informed by the history of previous outcomes. However, it is unclear to what extent these prior findings are applicable to the Rwandan context. The next trial, based on this study's findings, will therefore also include a safety endpoint. Because drug-resistance-preventing activity cannot be studied with our small study population, we investigate proxy endpoints that reflect these aims, such as the bactericidal effect in the first 2 weeks of treatment and AMK Cmax. At present, the two reference-standard endpoints for measuring the early bactericidal effect of a regimen are the amount of viable bacilli in sputum cultured on solid media and enumerated as CFUs, and the time-to-positivity in liquid media.[38] How well our proxy endpoints correlate with acquired resistance will need to be confirmed in larger multicountry cohorts powered for studying acquired resistance.

In conclusion, our study aims to contribute to the evidence base on how the resistance prevention activity of RR-TB treatment regimens can be strengthened. If indeed adding two doses of AMK during the first week is tolerable and protects against acquired resistance to BDQ and/or FQ, then both second-line core drugs would be safeguarded for future generations of TB patients. Future studies should also explore the use of alternative drugs like linezolid to strengthen the first weeks of RR-TB treatment and prevent resistance.

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Table S1: Treatment outcomes as shown in the ShORRT Master protocol

EVENT	DEFINITION
Favourable outcome	Composite outcome corresponding to the combination of "cured" + "treatment completed" (= treatment success) without recurrence over the 12-month follow-up period. Note: this outcome can also be defined as "recurrence-free cure"
Cured	A patient with bacteriologically confirmed MDR/RR-TB who has completed 9-12 months of treatment by 9/12-month regimen protocol without evidence of failure AND at least two consecutive cultures taken at least 30 days apart are negative at the end of the treatment and at least one month earlier.
Treatment Completed	A patient who completes 9-12 months of treatment by 9/12-month regimen protocol without evidence of failure BUT without bacteriological evidence (negative culture at the end of the treatment phase and at least one month earlier).
Treatment Failed	Treatment terminated or need for permanent change of the regimen protocol of at least two anti-TB drugs because of: • lack of sputum **culture** conversion after 4 months of treatment, or • bacteriological reversion of sputum **culture** after 5 months of treatment in a patient with previous culture conversion to negative, or • evidence of additional acquired resistance to drugs in the study, or • adverse drug reactions (ADRs) (leading to the change of at least two anti-TB drugs in the regimen)
Died	A patient who dies for any reason during the course of treatment.
Lost to follow-up	A patient whose treatment was interrupted for 2 consecutive months or more.
Not evaluated	A patient for whom no treatment outcome is assigned (this includes cases "transferred out" to another treatment unit and whose treatment outcome is unknown/can't be assessed)
Withdrawn	A patient is taken off the 9/12-month regimen for any reason other than treatment failure (for example, baseline second-line drug resistance, withdrawn patient informed consent or other reasons) and referred to the PMDT program for routine care.
Treatment Success	The sum of *cured* and *treatment completed.*
Recurrence	Cure or treatment completion followed by two consecutive positive cultures during post-treatment follow-up (without genotyping information on baseline and recurrent strain), or one positive culture with clinical signs and symptoms or radiographic deterioration.
Relapse	Recurrence in which isolates of the recurrent episode share the same genotype pattern with isolates of the first episode of MDR-TB.

Reinfection	Recurrence in which isolates of the recurrent episode and isolates of the first episode of MDR-TB have different genotype patterns.
Conversion (to negative)	Culture is considered to have converted to negative when two consecutive cultures taken at least 30 days apart are found to be negative. In such case, the specimen collection date of the first negative culture is used as the date of conversion. In case patients were culture negative at baseline, a negative culture result at month 4 may be considered as "initial conversion".
Reversion (to positive)	Culture is considered to have reverted to positive when after an initial conversion, two consecutive cultures taken at least 30 days apart are found to be positive. In case of patients who are culture negative at baseline, a positive culture result at month 4 may be considered as "initial conversion".
Treatment adherence	90% of the treatment doses were taken based on information in the treatment cards, measured over the entire treatment period.
Permanent disability	A combined outcome, using the modified Medical Research Council Dyspnoea scale (mMRC), based on which patients with a score above 2 are considered permanently disabled in terms of their pneumological function. In addition, all serious adverse events by system organ class that are not resolved at the end of treatment, should be summarised by treatment regimen. This is a measure of a programme's ability to start treatment promptly and treat patients effectively.
Serious Adverse Event (SAE)	Any untoward medical occurrence that may present in a TB patient during treatment with a pharmaceutical product, but which does not necessarily have a causal relationship with this treatment, which either leads to: ▪ death; ▪ a life-threatening experience; ▪ hospitalization or prolongation of hospitalization; ▪ persistent or significant disability; ▪ a congenital anomaly.