PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Snobre, Jihad; Gasana, Joel; Ngabonziza, Jean Claude Semuto; Cuella-Martin, Isabel; Rigouts, Leen; Jacobs, Bart Karl; de Viron, Emeline; Herssens, Natacha; Ntihumby, Jean Baptiste; Klibazayre, Annualithe; Ndayishimiye, Clement; Van Deun, Armand; Affolabi, Dissou; Merle, Corinne; Muvunyi, Claude; Sturkenboom, Marieke; Migambi, Patrick; de Jong, Bouke; Mucyo, Yves; Decroo, Tom

VERSION 1 – REVIEW

REVIEWER	Acuña-Villaorduña , Carlos Boston University
REVIEW RETURNED	18-Oct-2023

GENERAL COMMENTS	Snobre et al present a protocol study to evaluate the safety of two doses of amikacin administered during the first week of MDR TB treatment. The main objective of the study is to evaluate safety with a future aim to address prevention of emergence of resistance to BDQ. Amplification of resistance is an important outcome stated by WHO guidelines specially now with the availability of all oral BDQ containing regimens, however limited data is available on this important topic. In this context, the study is important, and the setting, inclusion and exclusion criteria are appropriate. Some comments below: 1. Historically, emergence of resistance has been associated with use of weaker TB regimens, I agree with the authors that BDQ EBA is delayed which could potentially lead to emergence of resistance. In this context, the accompanying drugs in the regimen are very important, pretomanid (PA) is also bactericidal and along with FQ tend to potentiate the effect of BDQ (Yamada et al PMID: 36165631). Thus the effect of amikacin may be less pronounced in patients receiving BPaLM, in fact, amplification of resistance was rarely seen in the BPaLM arm of TB PRACTECAL, It is possible however that amikacin may provide some protection when regimens that do not include pretomanid are being used, could the authors provide a background on the standard MDR regimen being used in Rwanda? As this could bring significant implications on the research aims. 2. The lack of a control group is a major limitation in the study, as both the primary and secondary outcomes could not be properly assessed and compared without a baseline group, the authors used an arbitrary 14% rate of expected AMK toxicity which seems excessive as the authors also pointed out. In STREAM2 trial, rates of vestibular toxicity and renal injury were 1% and 0%, despite

	patients using 8 weeks of kanamycin (albeit at a lower dose). In this context, the authors should strongly considering adding a control group to assess outcomes. 3. Line 181 and 183, Secondary outcomes: Any SAE associated with the use of AMK. Could the authors be more specific and clarify how will the differentiate SAE of AMK and other drugs in the regimen. Would they just focus on ototoxicity and nephrotoxicity as side effects? 4. Line 256, microbiological tests including baseline DST will be performed? Will MICs for bedaquiline be estimated? This is important but difficult as it requires MGIT or broth microdilution. If a control group is available, the authors could use BDQ MIC as surrogates markers of protection against amplification of resistance. Would the use of amikacin lead to lower MICs for bedaquiline at 2 weeks and 1-2 months of therapy, compared against a group that does not receive AMK? 5. Line 286, AMK blood levels: “Just before administration of AMK and 2 and 6 hrs after the administration of AMK on day 1 and 4”. On day 1, probably do NOT need to check AMK levels just before administration. 6. The authors chose AMK as drug of choice due to its bactericidal effect and previous studies showing some benefit (Reference 2, 12 and 13). I do agree that AMK is a good drug due to its post antibiotic effect, however I am not convinced AMK has great bactericidal effect as the authors state. The review by Reuter et all (PMID: 29037291) showed that there is limited evidence of the bactericidal activity of AG, in addition the study by Decroo et al (reference 3) showed that 2 months of kanamycin is not enough to prevent amplification of resistance. While I like the idea of using AMK in the study, I am not fully convinced by the authors rationale.

REVIEWER	Niward, Katarina Linköping University, Department of Biomedical and Clinical Sciences
REVIEW RETURNED	06-Jan-2024

GENERAL COMMENTS	This is a single-arm study to evaluate the safety of adding a 30 mg/kg amikacin (AMK) injection twice during the first week of RR-TB treatment as a protection for emergence of core second line drug resistance. The study is interesting with a new approach and some evidence in literature findings. Although, some points need to be considered or clarified before publication: General comment: The abstract and manuscript is well written, but the introduction is very long and should mainly focus on the background and rationale for the study including a brief overview of the objectives (structure according to the CONSORT). For instance, sections elaborating on details of study performance such as adding lidocaine to AMK, using FACES scale etc and information on Primary and Secondary Outcomes/specific measurement variables as well as how the sample size was determined, belong to the Method. Specific comments: 1. Consider adding information in the abstract that AMK is given Day 1 and Day 4. 2. If the authors have access to local surveillance data it would add depth to the paper to know the prevalence of BDQ, FQ and AMK resistance among patients in Rwanda with RR-TB.

	3. In Line 85-90 the authors mention the rate of BDQ-resistance (2.3%) developing during BDQ-containing MDR treatments without injectables compared to the much lower rate for development of RIF-resistance during first-line treatment. Adherence is crucial to any TB-regimens. It would be beneficial for the reader to know if that was taken into account or not (regarding BDQ-resistance) and further to know the rate of FQ-resistance developing during short-course MDR-treatment regimens, with or without injectables, for comparison. 4. Elaborate on the low level of acquired resistance found in TRUNCATE trial with the all-oral regimen of BDQ-LZD-INH-PZA-EMB (has the same concern regarding the delayed onset of action/long half-life of BDQ). 5. Line 103-105. The authors write that SLIs activity has a strong effect on the prevention of FQ-resistance. Explain more and compared with what? 6. Consider adding balanced information on the importance of adherence as this affects any regimen and is only reliably controlled/reported in prospective clinical trials such as the STREAM Stage 2 trial (reference 14) with notable low level of acquired resistance (Line 108-110). 7. I cannot find any information (or Supplementary material) on definitions (in some cases also the timepoint) that will be applied for “treatment outcome”, “end-of-treatment outcome”, “post-treatment outcome” and “after post-treatment outcome”. 8. I do not understand Line 169-170 “…after post-treatment follow-up” – what is the definition of post-treatment follow-up and why will this be assessed? 9. Is allergy to lidocaine (or similar drug class) not relevant as an exclusion criterion? 10. Is hereditary hearing loss within the family not relevant as an exclusion criterion? 11. The Exclusion criteria does not list resistance to SLI, but in the section on Laboratory procedures the authors state in Line 247-248 that “On admission, the patients provide sputa for….and exclusion of SLI resistance by Xpert XDR, at Kabutare hospital.”. The study is conducted at the Kabutare hospital where these point-of-care PCR-tests seem to be available. Undetected SLI resistance will affect the secondary treatment response outcome for STAKE (and expose patient that have no benefit from SLI-treatment). 12. How is overnight sputa performed/collected? Explanation is good in order for others to properly be able to repeat the study. 13. Is daily DOT or VOT applied during the first weeks of treatment or the entire intensive phase? How is adherence documented in the study? 14. In the section on Intervention description (Line 241-244) there is only information on stopping-criteria for nephrotoxicity. Are there no stopping-criteria for ototoxicity as audiometry is performed before the second administration of AMK? 15. What grading system (e.g. Common Terminology Criteria for Adverse Events) or definitions are used to grade other AEs (e.g. vertigo, tinnitus, vomiting, rash) beside nephrotoxicity and ototoxicity for which the authors already have provided templets in Table 3 and 4. 16. Line 352, consider to be more specific on “effectiveness”. Consider replacing with “treatment response endpoints” or something more informative.

	17. Line 361-362 is a bit unclear - does the identification by ID numbers mean that the study team will keep a securely stored code list? 18: Discussion Line 376 – reference 25 is the ZeNix-TB study referring to BPaL and pre-XDR cases. TB PRACTECAL study with BPaLM for MDR/RR-TB seems more relevant for this sentence or at least to be added. 19. Do the authors have historical surveillance data of the incidence for ototoxicity and/or nephrotoxicity during SLI-containing TB regimen in Rwanda? 20. Discuss the possible influence on the hypothesis concerning the fact that aminoglycosides have poor tissue penetration. 21. Line 432-444 contain several sentences that would benefit from being simplified and shorter. A couple of words are missing for full understanding e.g. “of” in the end of Line 440. 22. Line 472 – I cannot find any author corresponding to CSM? Table 1: • Are there any concerns with 30mg/kg AMK to people with high BMI (>30) due to the ADME properties of AMK? Have the authors considered to apply adjusted body weight instead of total body weight for this group? Table 2: • What does the empty row with only “µ” in the first cell mean? • Please clarify when staff - by actively asking the patient or by using a questionnaire - assess AEs in the schedule. Provide a separate row for this type of AE. • Informed consent is based on meeting the inclusion criteria, but if SLI resistance is an exclusion criterion (see comment nr 11) Xpert XDR may be added to the necessary assessments listed in Table 2.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Carlos Acuña-Villaorduña, Boston University

Comments to the Author:

Snobre et al present a protocol study to evaluate the safety of two doses of amikacin administered during the first week of MDR TB treatment. The main objective of the study is to evaluate safety with a future aim to address prevention of emergence of resistance to BDQ. Amplification of resistance is an important outcome stated by WHO guidelines specially now with the availability of all oral BDQ containing regimens, however limited data is available on this important topic. In this context, the study is important, and the setting, inclusion and exclusion criteria are appropriate. Some comments below:

1. Historically, emergence of resistance has been associated with use of weaker TB regimens, I agree with the authors that BDQ EBA is delayed which could potentially lead to emergence of resistance. In this context, the accompanying drugs in the regimen are very important, pretomanid (PA) is also bactericidal and along with FQ tend to potentiate the effect of BDQ (Yamada et al PMID: 36165631). Thus the effect of amikacin may be less pronounced in patients receiving BPaLM, in fact, amplification of resistance was rarely seen in the BPaLM arm of TB PRACTECAL, It is possible however that amikacin may provide some protection when regimens that do not include pretomanid are being used, could the authors provide a background on the standard MDR regimen being used in Rwanda? As this could bring significant implications on the research aims.

   The use of the BPaL(M) regimen has indeed demonstrated a low amplification of bedaquiline resistance in clinical trial settings, while data from programmatic use is only now becoming available. Recent studies have shown that lineage 1, accounting for 28% of the strains in Africa and Asia, was less susceptible than lineages 2, 3, 4 and 7 of M. tuberculosis, resulting in a 99th percentile of 2 mg/L for lineage 1 compared with 0.5 mg/L for the remaining M. tuberculosis lineages, with provisional critical concentration of 1 mg/L for MGIT (PMID: 35260883, PMID: 38334384). Also linezolid resistance is being increasingly reported (PMID: 38000314), with many countries lacking the capacity to conduct pretomanid and linezolid DST. This may weaken the BPaLM regimen especially during the first weeks, also considering the delayed onset of bedaquiline action. In Rwanda, the all-oral short regimen (4-6 BDQ(6m)-Lfx-Cfz-Z-E-Hh-Eto/5 Lfx-Cfz-Z-E) is currently used to treat MDR-TB patients, while BPaLM will be implemented in July 2024. The composition of the current all-oral short regimen has been specified in the paper for clarity. In the future Phase 3 study we plan to incorporate AMK to strengthen the bedaquiline- containing regimens including BPaL(M).

- 2. The lack of a control group is a major limitation in the study, as both the primary and secondary outcomes could not be properly assessed and compared without a baseline group, the authors used an arbitrary 14% rate of expected AMK toxicity which seems excessive as the authors also pointed out. In STREAM2 trial, rates of vestibular toxicity and renal injury were 1% and 0%, despite patients using 8 weeks of kanamycin (albeit at a lower dose). In this context, the authors should strongly considering adding a control group to assess outcomes.

  Since our primary objective is to investigate adverse events that are likely or definitively related to the use of amikacin, and given that the standard regimen does not include amikacin, we chose to conduct a single-arm study, thus without control arm. For the secondary outcomes, while a formal inclusion of a control group is not planned, the Stake study is nested within a larger study named ShORRT, which evaluates the effect of the 9 months short-all oral regimen containing bedaquiline (BDQ). This allows for comparison of bacteriological treatment response outcomes between regimens with and without the inclusion of amikacin in a planned sub-study. We added a sentence in the discussion regarding this matter. A post-treatment evaluation has been organized in Rwanda in 2021 on long-term outcomes among former DR-TB patients who had successfully completed second line TB treatment in Rwanda between 2010 and 2017

(266 patients in total) and found that 21% of the patients showed moderate to profound hearing loss; these are unpublished data and couldn't be included yet in the protocol. Also a review indicated that interruptions due to amikacin accounted for 10.2% [6.3–16.0] (Lan et al., 2020), a figure also cited in WHO guidelines. Considering this might be an underestimation—as some grade 3 adverse events (AEs) may have been overlooked, and other studies report a far higher incidence of severe ototoxicity than 10% (Tahseen et al., 2021)—we opted for a higher cutoff of 14%. Our hypothesis is that none of the 20 patients enrolled will experience a serious adverse event, with the upper bound of the 95% confidence interval remaining below 14%.

3. Line 181 and 183, Secondary outcomes: Any SAE associated with the use of AMK. Could the authors be more specific and clarify how will the differentiate SAE of AMK and other drugs in the regimen. Would they just focus on ototoxicity and nephrotoxicity as side effects?
   While our primary endpoint is any grade 3-4 adverse event during the first 2 weeks of treatment, assessed as likely or definitely related to the use of AMK; while our focus will primarily be on ototoxicity and nephrotoxicity monitoring, side effects known to be caused by the use of amikacin, we will also document all other adverse events; we agree with the reviewer that these may be less specific; the determination of the relationship between the adverse event and the drug, will be made by the clinician on site as done for other studies. This assessment will take into account various factors, including the onset of symptoms, concurrent pathologies, and the use of other medications.

4. Line 256, microbiological tests including baseline DST will be performed? Will MICs for bedaquiline be estimated? This is important but difficult as it requires MGIT or broth microdilution. If a control group is available, the authors could use BDQ MIC as surrogates markers of protection against amplification of resistance. Would the use of amikacin lead to lower MICs for bedaquiline at 2 weeks and 1-2 months of therapy, compared against a group that does not receive AMK?
   We thank the reviewer for the suggestion. DTS are indeed performed, while it's not possible to modify the current protocol, we will include BDQ MIC as predictor for resistance amplification in the next multi-country study evaluating effectiveness of amikacin to protect BDQ containing regimens.

5. Line 286, AMK blood levels: "Just before administration of AMK and 2 and 6 hrs after the administration of AMK on day 1 and 4". On day 1, probably do NOT need to check AMK levels just before administration.
   Indeed, before the second administration only, we changed this line in the text to clarify.

6. The authors chose AMK as drug of choice due to its bactericidal effect and previous studies showing some benefit (Reference 2, 12 and 13). I do agree that AMK is a good drug due to its post antibiotic effect, however I am not convinced AMK has great bactericidal effect as the authors state. The review by Reuter et all (PMID: 29037291) showed that there is limited evidence of the bactericidal activity of AG, in addition the study by Decroo et al (reference 3) showed that 2 months of kanamycin is not enough to prevent amplification of resistance. While I like the idea of using AMK in the study, I am not fully convinced by the authors rationale.

We agree with the reviewer that there is limited evidence for the bactericidal effect of AMK. However, the preventing effect of SLI on acquired drug resistance has been reported in several studies. The study (PMID: 32866193) analyzing the effect of using 2 months of kanamycin instead of the standard 4(+) months on recurrence and fluoroquinolone acquired drug resistance in patients treated with a gatifloxacin-based STR in Bangladesh showed that two 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.

The studies led by CDC Atlanta (PMID: 25057101, PMID: 26508515) have shown that the treatment regimens with active SLI (compared to those without, e.g. due to baseline resistance to SLI) had a strong effect on the prevention of FQ-resistance acquisition compared to regimens not approved by the Green Light Committee. This study (PMID: 25057101) also showed that baseline resistance to the SLI had the greatest impact on the risk of acquired XDR tuberculosis (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 tuberculosis was 2.4%. With baseline resistance to an FQ, the risk of acquired XDR was 16.7%. With baseline SLI resistance, the risk of acquired XDR was 36.8% to 46.0%, depending on the specific drug. 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. Together with their pharmacokinetic and pharmacodynamic characteristics this makes them a good candidate to strengthen the first week of all-oral STR. Since the efficacy of AMK is correlated with the peak serum concentration (or Cmax) over minimum inhibitory concentration (MIC), we use AMK high dose at 30 mg/kg only in the first week of treatment, aiming to complement the delayed onset of bedaquiline bactericidal effect. We updated the protocol to expand on the reasons for choosing SLIDs as strengthening drug for the first week of treatment.

Reviewer 2

This is a single-arm study to evaluate the safety of adding a 30 mg/kg amikacin (AMK) injection twice during the first week of RR-TB treatment as a protection for emergence of core second line drug resistance. The study is interesting with a new approach and some evidence in literature findings. Although, some points need to be considered or clarified before publication:

General comment: The abstract and manuscript is well written, but the introduction is very long and should mainly focus on the background and rationale for the study including a brief overview of the objectives (structure according to the CONSORT). For instance, sections elaborating on details of study performance such as adding lidocaine to AMK, using FACES scale etc and information on Primary and Secondary Outcomes/specific measurement variables as well as how the sample size was determined, belong to the Method.

We thank the reviewer for the suggestion, we shortened the introduction as indicated.

Specific comments:

1. Consider adding information in the abstract that AMK is given Day 1 and Day 4.

This is already specified in the abstract (line 31).

2. If the authors have access to local surveillance data it would add depth to the paper to know the prevalence of BDQ, FQ and AMK resistance among patients in Rwanda with RR-TB.

In a study by Habimana-Mucyo et al. (PMID: 35653710), the prevalence of rifampicin-resistant tuberculosis (RR-TB) was evaluated among all bacteriologically confirmed pulmonary TB patients notified from July 1, 2019, to June 30, 2020, in Rwanda. The prevalence of RR-TB was calculated among those with DST results. It was found to be 1.4% for new TB cases and 4.9% for previously treated cases. Out of 73 patients with RR-TB, 49 had results for isoniazid DST, and 28 (57.1%) were diagnosed with multidrug-resistant TB (MDR-TB), indicating resistance to both rifampicin and isoniazid. Second-line DST results were available for 48 (65.8%) of RR-TB cases. No resistance to fluoroquinolones was observed. However, one patient exhibited RR-TB with resistance to all second-line injectable agents, and five patients showed RR-TB resistance to ethionamide. We summarized these data and added in the study protocol.

3. In Line 85-90 the authors mention the rate of BDQ-resistance (2.3%) developing during BDQ containing MDR treatments without injectables compared to the much lower rate for development of RIF-resistance during first-line treatment. Adherence is crucial to any TB-regimens. It would be beneficial for the reader to know if that was taken into account or not (regarding BDQ-resistance) and further to know the rate of FQ-resistance developing during short-course MDR-treatment regimens, with or without injectables, for comparison.

Given recent data on acquired BDQ resistance in programmatic settings we updated the text at line 84-90; we expand on rates of FQ-resistance under injectable-containing regimens in response to point 5 (see below). Unfortunately, we couldn't find studies reporting rates of acquired FQ resistance under bedaquiline-containing regimens. Neither did we find studies showing the association between acquired BDQ resistance and adherence.

Current text:

"The recent TRUNCATE study showed that BDQ may be more potent than even high dose rifampicin, allowing treatment shortening to two months (5). Nevertheless, recent studies have shown that all-oral MDR/RR-TB regimens, including BDQ and a FQ but without SLIs, are associated with acquired resistance. Specifically, these studies report a bacteriologically

adverse outcome with acquired BDQ resistance of around 2.3%, which is much higher than the rates of acquired rifampicin resistance with the first-line regimen (0.1%).”

Updated text:

“Bedaquiline acquired resistance is being increasingly reported in programmatic settings. In 2021 Ismail et al had reported a rate of acquired BDQ resistance of around 2.3% under bedaquiline containing regimens (although adherence data were not reported). Other reports from Pakistan (PMID: 31262765) and Moldova (PMID: 34503982) showed that six (20%) of 30 and four (15%) of 26 patients acquired bedaquiline resistance under bedaquiline 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 in for all patients included in the ShORRT master study adherence will be reported."

4. Elaborate on the low level of acquired resistance found in TRUNCATE trial with the all-oral regimen of BDQ-LZD-INH-PZA-EMB (has the same concern regarding the delayed onset of action/long half-life of BDQ).

We agree with the reviewer that the TRUNCATE trial showed low rates of bedaquiline acquired resistance; however TRUNCATE only included patients with susceptible TB, initially excluded patients with a high bacillary load, and used a regime where INH could provide resistance preventing activity, while studies on RR-TB patients under bedaquiline-containing regimens reported much higher resistance rates, as indicated in point 3. Moreover, resistance acquisition with the TRUNCATE regimen in routine care, with a less selected study population, and with less stringent follow-up remains unknown. Since TRUNCATE includes rifampicin-susceptible patients, the reference is probably not well-placed, we removed it from the introduction section where it was mentioned.

5. Line 103-105. The authors write that SLIs activity has a strong effect on the prevention of FQ resistance. Explain more and compared with what?

We updated the text expanding on studies reporting FQ acquired resistance under SLI activity; however we couldn’t find relevant studies reporting rates of acquired FQ resistance under BDQ-containing regimens.

Current text line 103-110:

A Damien Foundation Bangladesh study (3) and another study led by CDC Atlanta (12)(13) have shown that the activity of SLIs has a strong effect on the prevention of FQ-resistance acquisition. Together with their pharmacokinetic and pharmacodynamic characteristics this makes them the ideal candidate to strengthen the first week of all-oral STR. Notably, SLIs protected BDQ better than linezolid in Pakistan (7). 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 (14).

Updated text:

A study (PMID: 32866193) analyzing the effect of using 2 months of kanamycin instead of the standard 4(+) months on recurrence and fluoroquinolone acquired drug resistance in patients treated with a gatifloxacin-based STR in Bangladesh showed that two 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.

The studies led by CDC Atlanta (PMID: 25057101, PMID: 26508515) have shown that the treatment regimens with active SLI (compared to those without, e.g. due to baseline resistance to SLI) had a strong effect on the prevention of FQ-resistance acquisition compared to regimens not approved by the Green Light Committee. This study (PMID: 25057101) also showed that baseline resistance to the SLI had the greatest impact on the risk of acquired XDR tuberculosis (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 tuberculosis was 2.4%. With baseline resistance to an FQ, the risk of acquired XDR was 16.7%. With baseline SLI resistance, the risk of acquired XDR was 36.8% to 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 bedaquiline was used either with or without SLIDs acquired bedaquiline resistance was significantly more frequent when SLIDs did not protect bedaquiline [53.8% (7/13) versus 7.7% (1/13); OR 9.6; 95% CI 1.3–70.5] and in patients previously treated with a SLID-containing second-line regimen [58.3% (7/12) versus 7.1% (1/14); OR 12.3; 95% CI 1.6–92.0] (PMID: 33258921). 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.

6. Consider adding balanced information on the importance of adherence as this affects any regimen and is only reliably controlled/reported in prospective clinical trials such as the STREAM Stage 2 trial (reference 14) with notable low level of acquired resistance (Line 108-110).

We thank the reviewer for the suggestion, please see answer at point 3.

7. I cannot find any information (or Supplementary material) on definitions (in some cases also the timepoint) that will be applied for “treatment outcome”, “end-of-treatment outcome”, “posttreatment outcome” and “after post-treatment outcome”.

Definition of treatment outcomes are the same described in the master ShORRT protocol evaluating effectiveness of all-oral BDQ-containing short regimen; all Stake patients are nested in the ShORRT study. We added a supplementary file with treatment outcomes definitions as reported in the master protocol published by WHO.

8. I do not understand Line 169-170 “…after post-treatment follow-up” – what is the definition of post-treatment follow-up and why will this be assessed?

Post-treatment follow-up for 12 months after treatment is needed to assess outcomes such as early relapse. It consists of a follow-up ambulatory visits from the clinician with symptom assessments and eventual additional tests if relapse is suspected at 6 months and 12 months after treatment end. We added a sentence to explain, this is also described more into details in the ShORRT master protocol.

9. Is allergy to lidocaine (or similar drug class) not relevant as an exclusion criterion?

This was not specifically mentioned as an exclusion criterion; however, general allergies are included as part of the routine history on the treatment card. For the next study, we will consider explicitly stating this as an exclusion criterion.

10. Is hereditary hearing loss within the family not relevant as an exclusion criterion?

While any hearing loss at screening is an exclusion criterion, a family history of hereditary hearing loss is not; it will be considered for the next study evaluating the effectiveness of the intervention in preventing drug resistance.

11. The Exclusion criteria does not list resistance to SLI, but in the section on Laboratory procedures the authors state in Line 247-248 that “On admission, the patients provide sputa for….and exclusion of SLI resistance by Xpert XDR, at Kabutare hospital.”. The study is conducted at the Kabutare hospital where these point-of-care PCR-tests seem to be available. Undetected SLI resistance will affect the secondary treatment response outcome for STAKE (and expose patient that have no benefit from SLI-treatment).

Resistance to SLI is indeed part of the exclusion criteria, explicitly added to the exclusion criteria in the text.

12. How is overnight sputa performed/collected? Explanation is good in order for others to properly be able to repeat the study.

Since we exceeded the word count, we added a reference for the overnight sputum collection as it is implemented in the TB program in Rwanda in the laboratory procedures section in the methods.

13. Is daily DOT or VOT applied during the first weeks of treatment or the entire intensive phase? How is adherence documented in the study?

In the first weeks of treatment, patients are treated at the hospital with directly observed therapy. Patients clinically stable with at least one culture negative are sent back home for ambulatory treatment. Directly observed therapy is ensured at the health facility near the patient's home. This is described in the Methods – study setting section.

14. In the section on Intervention description (Line 241-244) there is only information on stopping criteria for nephrotoxicity. Are there no stopping-criteria for ototoxicity as audiometry is performed before the second administration of AMK?

A Standard Operating Procedure is in place for ototoxicity monitoring. Briefly, if an increase in hearing loss greater than 20dB at any frequency relative to baseline values is detected during the Day 3 examination after the first injection, the patient will not receive the second dose of amikacin and will be referred to the Kabutare ENT specialists for urgent further examination. We clarified in the text.

15. What grading system (e.g. Common Terminology Criteria for Adverse Events) or definitions are used to grade other AEs (e.g. vertigo, tinnitus, vomiting, rash) beside nephrotoxicity and ototoxicity for which the authors already have provided templets in Table 3 and 4.

Indeed, the Common Terminology Criteria for Adverse Events is used to grade adverse events, excluding nephrotoxicity and ototoxicity. A sentence has been added to the text for clarification.

16. Line 352, consider to be more specific on "effectiveness". Consider replacing with "treatment response endpoints" or something more informative.

Done, replaced with "treatment response endpoints".

17. Line 361-362 is a bit unclear - does the identification by ID numbers mean that the study team will keep a securely stored code list?

Indeed the register correlating patient ID with the patient's demographic data is kept in a secured locked cabinet in the clinical staff office. We clarified in the text.

18: Discussion Line 376 – reference 25 is the ZeNix-TB study referring to BPaL and pre-XDR cases. TB PRACTECAL study with BPaLM for MDR/RR-TB seems more relevant for this sentence or at least to be added.

Done.

19. Do the authors have historical surveillance data of the incidence for ototoxicity and/or nephrotoxicity during SLI-containing TB regimen in Rwanda?

A post treatment evaluation has been organized in Rwanda in 2021 on long-term outcomes among former DR-TB patients who had successfully completed second line TB treatment in Rwanda between 2010 and 2017 (266 patients in total) and found that 21% of the patients showed moderate to profound hearing loss; these are unpublished data and couldn't be included yet in the protocol.

20. Discuss the possible influence on the hypothesis concerning the fact that aminoglycosides have poor tissue penetration.

As discussed in the introduction 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). A hollow-fiber 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 (PMID: 30496466). Considering poor penetration of SLIs in lung tissue, this translates into a serum Cmax/MIC ratio of 75 (PMID: 30496466). With two doses of 30 mg/kg, we aim to obtain the highest efficacy also taking into account the poor penetration in lung tissues.

21. Line 432-444 contain several sentences that would benefit from being simplified and shorter. A couple of words are missing for full understanding e.g. "of" in the end of Line 440.

Done, updated text below:

"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 two 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 (36). How well our proxy endpoints correlate with acquired resistance

will need to be confirmed in larger multi-country cohorts powered for studying acquired resistance."

22. Line 472 – I cannot find any author corresponding to CSM?

CSM corresponds to C.S Merle.

Table 1: • Are there any concerns with 30mg/kg AMK to people with high BMI (>30) due to the ADME properties of AMK? Have the authors considered to apply adjusted body weight instead of total body weight for this group?

According to the protocol, a maximum of 3000 mg of AMK is administered. To avoid underdosing in individuals with a high BMI, the application of adjusted body weights could indeed be considered. This approach may be incorporated into future studies using high-dose AMK.

Table 2: • What does the empty row with only "μ" in the first cell mean?

This may be a format confusion; the symbol μ pertains to the 'bacteriological solid and liquid culture' row directly above.

• Please clarify when staff - by actively asking the patient or by using a questionnaire - assess AEs in the schedule. Provide a separate row for this type of AE.

As described in the Methods section on Safety Assessment and Reporting, patients undergoing directly observed therapy are monitored for any potential adverse events (AEs) according to the schedule of events and through passive reporting. The adverse events are documented on the treatment cards and managed by the clinical staff. The clinical staff also completes a cumulative adverse event report in the electronic database on Day 14, and then monthly. We have added a row in the table to reflect these formal assessments and have clarified this in the description of the table.

• Informed consent is based on meeting the inclusion criteria, but if SLI resistance is an exclusion criterion (see comment nr 11) Xpert XDR may be added to the necessary assessments listed in Table 2.

Done, we added a row in the table for Xpert XDR at screening.

VERSION 2 – REVIEW

REVIEWER	Acuña-Villaorduña , Carlos Boston University
REVIEW RETURNED	30-May-2024

GENERAL COMMENTS	The authors have responded properly all my previous comments.