Four-Month High-Dose Rifampicin Regimens for Pulmonary Tuberculosis

Amina Jindani[1†], Daniel Atwine[2,3], Daniel Grint[4], Boubacar Bah[5], Jack Adams[1], Eduardo Rómulo Ticona[6], Bhabana Shrestha[7], Tefera Agizew[8], Saeed Hamid[9], Bushra Jamil[9], Adolf Byamukama[2], Keneth Kananura[2], Ivan Mugisha Taremwa[2], Maryline Bonnet[2,10], Lansana Mady Camara[5], Oumou Younoussa Bah-Sow[5], Kindy Sadjo Bah[5] Nene Mamata Bah[5], Maimouna Sow[5], César Eduardo Ticona Huaroto[6], Raquel Mugruza Pineda[6], Bijesh Tandukar[7], Bijendra Bhakta Raya[7], Neko Shrestha[7], Anikie Mathoma[8], Unami P. Mathebula- Modongo[8] , Joyce Basotli[8], Muhammad Irfan[9], Dilshad Begum[9], Ammara Muzammil[9], Imran Ahmed[9], Rumina Hasan[9], Marcos V Burgos[11], Faisal Sultan[12], Mariam Hassan[12], Iqra Masood[12], Claire Robb[1], Jonathan Decker[13], Sisa Grubnic[14], Philip D. Butcher[1], Adam Witney[1], Jasvir Dhillon[1], Tulika Munshi[1], Katherine Fielding[4], and Thomas S Harrison[1,14,15†], on behalf of The Rifashort Study Group.

[1]Institute for Infection and Immunity, St. George's, University of London, United Kingdom

[2]Epicentre/Mbarara Research Base, Mbarara. Uganda

[3]Mbarara University of Science and Technology, Mbarara, Uganda.

[4]London School of Hygiene and Tropical Medicine, London, United Kingdom

[5] Centre Hospitalier Universitaire Ignace Deen, Conakry, Guinea

[6]Hospital Nacional Dos de Mayo, Universidad Nacional Mayor de San Marcos, Lima,Peru

[7]German Nepal TB Project (GENETUP)/ Nepal Anti TB Association (NATA), Kathmandu, Nepal

[8]University of Botswana, Gaborone, Botswana

[9]Aga Khan University Hospital, Karachi, Pakistan

[10]University of Montpellier, TransVIHMI, IRD, INSERM, Montpellier France

[11]Division of Infectious Diseases, University of New Mexico, Albuquerque, NM, USA

[12]Shaukat Khanum Research Centre and Cancer Hospital, Lahore

[13]Department of Respiratory Sciences, University of Leicester, Leicester, UK

[14]Clinical Academic Group in Infection and Immunity, St George's University Hospitals NHS Foundation Trust, London, UK

[15]MRC Centre for Medical Mycology, University of Exeter, Exeter, UK

†Corresponding Authors

Short Title: RIFASHORT Trial

Key Words: Tuberculosis; Pulmonary; Rifampicin; Four months; High dose, Randomized Controlled Trial

Word Count: 3630

ABSTRACT

Background:

Shorter, but effective, tuberculosis treatment regimens would be of value to the tuberculosis treatment community. High-dose rifampicin has been associated with more rapid and secure lung sterilization and may enable shorter tuberculosis treatment regimens.

Methods:

We randomly assigned adults with newly diagnosed, sputum GeneXpert-positive, rifampicin-susceptible pulmonary tuberculosis to one of three regimens: a standard 6-month control regimen (control); an otherwise similar 4-month regimen in which rifampicin was dosed at 1200 mg/d (SR1); or a 4-month regimen in which rifampicin was dosed at 1800 mg/d (SR2). Sputum specimens for microscopy and culture were collected at regular intervals. The primary end point was a composite of treatment failure and relapse in participants who were sputum smear-positive at baseline. The non-inferiority margin was 8 percentage points. Using a sequence of ordered hypotheses, non-inferiority of SR2 was tested first.

Results:

Between January 2017 and December 2020, 672 patients were enrolled in centers in Uganda, Guinea, Peru, Nepal, Botswana, and Pakistan. The primary modified intention-to-treat population included 191 participants in the control arm, 192 in the SR1 arm, and 195 in the SR2 arm. Non-inferiority was not demonstrated. Favorable responses rates were 93%, 90% and 87% in the control, SR1, and SR2 arms, respectively, for a country-adjusted absolute risk difference of 6.3 percentage points (90% confidence interval: 1.1 to 11.5) comparing SR2 with control. The

proportion of participants experiencing a grade 3 or 4 adverse event was 4.0%, 4.5%, and 4.4% in the control, SR1, and SR2 arms, respectively.

Conclusions:

Four-month high-dose rifampicin regimens did not have dose-limiting toxicities or side-effects but failed to meet non-inferiority criteria when compared with the standard 6-month control regimen for treatment of tuberculosis.

Funded by the MRC/Wellcome Trust/DFID Joint Global Health Trials Scheme; Trial registration number: NCT02581527

BACKGROUND

Worldwide, an estimated 10 million people develop tuberculosis each year, and 1.4 million die from the disease[1]. The fact that cure is not always achieved in routine treatment may, in part, be due to patients failing to adhere to the current 6-month regimen recommended by the World Health Organization (WHO). Reducing treatment to 4 months may improve adherence and increase treatment completion and cure rates. In addition, reducing the duration of treatment would likely lessen the inconvenience and economic costs of treatment for patients[2].

Rifampicin is the cornerstone of current therapy due to its ability to kill not only the *Mycobacterium tuberculosis* undergoing rapid metabolism, but also the persistent mycobacteria thought to be responsible for most relapses[3]. The current standard dose of rifampicin (10 mg/kg) is the minimally effective dose historically selected based on pharmacokinetic, toxicity, and cost considerations[4]. However, subsequent animal model studies have demonstrated that high-dose rifampicin leads to more rapid sterilization and, in particular, a dose-dependent eradication of persistent mycobacteria[5], allowing for shorter treatment duration without relapse[5-9]. Furthermore, randomized controlled trials in patients receiving higher doses of rifampicin (15 – 35 mg/kg) have indicated higher culture conversion rates with no increase in serious adverse events[10-18].

Rifapentine has also been evaluated for the treatment of pulmonary tuberculosis. In the recent trial conducted by the Centers for Disease Control and Prevention's Tuberculosis Trials Consortium (TBTC) and the National Institutes of Health AIDS Clinical Trials Group (TBTC Study

31/A5349), a 4-month regimen containing daily rifapentine (at 1200 mg) and moxifloxacin met non-inferiority criteria, with outcomes comparable to the standard 6-month regimen[19]. However, rifampicin has some significant advantages over rifapentine as it has lower protein binding and better distribution into cavitary contents[20]. Furthermore, rifampicin is inexpensive, universally available, and used by national programs, suggesting few barriers would exist to implementation if a 4-month rifampicin-based regimen proved effective.

Thus, the objective of the RIFASHORT trial was to evaluate the efficacy and safety of a higher dose of rifampicin, 1200 mg/d or 1800 mg/d, with the aim of achieving evidence supporting its use for more rapid and secure sterilization of the lungs and a reduction of treatment duration to 4 months.

METHODS

Trial Oversight

This open-label, phase III, randomized, controlled, non-inferiority trial was carried out within the framework of the International Consortium for Trials of Chemotherapeutic Agents in Tuberculosis (INTERTB). The trial was sponsored and implemented by St. George's, University of London with statistical analysis by the London School of Hygiene and Tropical Medicine (LSHTM), in collaboration with institutions in sub-Saharan Africa, South America and South Asia. The trial protocol (available with this article at evidence.nejm.org) was approved by the LSHTM Research Ethics Committee as well as institutional and national ethics and regulatory authorities representing all participating sites and countries. Written informed consent was

obtained from participants. An independent data monitoring committee oversaw the trial. The trial funder, suppliers and drug manufacturers had no role in the trial design, data collection, analysis, interpretation, or manuscript presentation. AJ, DG, JA and TH wrote the first draft of the manuscript. The authors vouch for the accuracy and completeness of the data, for adherence to the protocol, and for the decision to publish.

Participants

Participants were recruited from three African sites: University of Botswana, Gaborone, Botswana; Epicentre, Mbarara, Uganda; Hospital National Ignace Deen, Conakry, Guinea; two sites in South Asia: GENETUP-NATA, Kathmandu, Nepal; Aga Khan University Hospital, Karachi, Pakistan; and one site in South America: Hospital Nacional Dos de Mayo, Lima, Peru.

Eligible participants were newly diagnosed (by sputum Xpert MTB/RIF nucleic acid amplification assay) with drug-susceptible pulmonary tuberculosis, aged 18 years or older, and had undergone no more than 1 week of treatment. Human immunodeficiency virus (HIV)-positive individuals, those with pre-existing liver disease, i.e., alanine transaminase (ALT) > five times the upper limit of normal, creatinine clearance < 30 mls/min, and diabetes mellitus were ineligible to participate.

Participants were excluded from efficacy analyses after randomization if they were found to have drug resistance to rifampicin and/or isoniazid (by Genotype MTBDR line probe assays

[Hain Lifescience] or direct susceptibility testing). Complete eligibility criteria can be found in the trial protocol, section 3.

Randomization and Treatment

Participants were randomly assigned in a 1:1:1 ratio, stratified by site, to one of three regimens: the control regimen (CR), study regimen 1 (SR1), and study regimen 2 (SR2). A randomized allocation sequence was generated for each trial center using blocks of varying size. Sealed opaque envelopes containing the treatment allocation slips were held by the pharmacist or nurse at each site. Staff at St. George's and participating laboratories were unaware of treatment assignment throughout the trial.

The control regimen was the WHO-recommended standard 6-month treatment for drug-susceptible pulmonary tuberculosis consisting of daily rifampicin (10mg/kg) and isoniazid, with ethambutol and pyrazinamide for the first 2 months (2HRZE/4HR); SR1 consisted of 4 months of rifampicin at 1200mg daily and isoniazid, with ethambutol and pyrazinamide for 2 months ($2EHR_{1200}Z/2HR_{1200}$); SR2 was identical to SR1 but with rifampicin at 1800mg daily ($2EHR_{1800}Z/2HR_{1800}$). Treatment was administered 7 days per week and was monitored as noted below. Additional details are provided in the protocol and in the Supplementary Appendix, page 6.

Trial Procedures

Participants were followed up for 18 months from randomization, apart from those recruited in the final 6 months of recruitment. In the final 6 months of recruitment, follow-up was decreased on a monthly basis until, in the final month, follow-up was for the minimum of 12 months, in order to prolong the enrollment period of the trial[21].

Participants were monitored and samples collected according to the assessment schedule (Table S6). Blood samples were collected for biochemical analysis, including liver function tests every two weeks for the first 6 weeks and monthly thereafter until one month post treatment phase. Sputum samples were collected monthly from 2 to 12 months, and then at 15 and 18 months. Standard mycobacteriology procedures at all trial sites were performed according to trial guidelines (bacteriological guidelines, available with this article at evidence.nejm.org) which included sputum Xpert MTB/RIF or Xpert MTB/RIF ultra (when this became available at sites) nucleic acid amplification tests, smear microscopy, and mycobacterial cultures on solid media using LJ or Ogawa slants, prepared or commercially sourced according to the site's usual practice. Drug susceptibility testing was performed for isoniazid and rifampicin on *M. tuberculosis* isolates at baseline and on any positive cultures after week 8. For patients in whom tuberculosis recurred, all positive cultures were shipped to St George's, where cultures were regrown, and DNA extracted. Whole genome sequencing was carried out to distinguish between relapse and a reinfection[22]. Exogenous reinfection was identified if the number of single nucleotide polymorphism (SNP) differences between pre- and post-treatment isolates was > 100.

Adherence to trial medication was monitored through directly observed therapy (DOT), supervised in the clinic and at home by a domiciliary treatment monitor (DTM). A time allowance was in place for making up missed doses within two weeks of completing the intensive phase, and four weeks of completing the continuation phase. In addition, allowance was made for treatment extension for participants following the hepatotoxicity drug reintroduction schedule in the protocol. In such cases, the endpoint review committee determined whether adequate treatment had been taken. Details of the definition of adequate treatment are reported in the statistical analysis plan, section 4.1.8.

Baseline chest x-ray images were read centrally, and categorized based on extent of disease and cavitation (Supplementary Appendix, page 9) by an independent expert at St George's Hospital, London, who was blinded to the allocated treatment.

Analysis Populations

The primary efficacy analysis followed the modified intention-to-treat (mITT) principle common in trials of tuberculosis therapy, whereby late exclusions due to drug resistance are removed from the analysis set. The primary analysis set included all microscopy-positive participants, defined as positive on culture and sputum smear at baseline (mITT-M). Given increasing use of Xpert MTB/RIF nucleic acid amplification assays for diagnosis of tuberculosis, we also recruited participants who were Xpert MTB/RIF positive, but microscopy smear negative, and, to increase the generalizability of the findings, defined a secondary analysis population including all Xpert MTB/RIF positive participants (mITT-All). Two further per protocol (PP) secondary analysis sets

were defined (PP-M; PP-All). The PP analysis sets differ from mITT in that any participant who did not complete an adequate course of treatment due to loss to follow-up or withdrawal was considered unassessable. Safety was assessed in all randomized participants receiving at least one dose of treatment. Full details of the analysis populations are given in the statistical analysis plan, section 5.

Primary Outcome

The primary efficacy outcome was the proportion of baseline sputum smear-positive patients with an unfavorable composite outcome measured by the end of follow-up. This period was 18 months for the majority of participants (83.6%), between 12 and 18 months for 14.3% and a minimum of 12 months for 2.1%. Unfavorable outcomes were defined as any of the following: death during the treatment phase or post-treatment death where tuberculosis was considered a plausible cause by the endpoint review committee; loss to follow-up during the treatment phase; participant withdrawal during treatment; permanent change in treatment due to an adverse event; two consecutive positive cultures after completing treatment; or re-treatment. Participants attending the final trial visit having maintained culture negative status, not otherwise classified as unfavorable, were classified as a favorable outcome. Participants who were lost to follow-up or withdrew from the trial while culture negative, those who died after completing treatment with no plausible link to tuberculosis, and those with evidence of exogenous reinfection were classified as unassessable. These definitions are consistent with those used in prior trials[23,24]. Full details are given in the protocol and statistical analysis plan, section 4.2.

The primary safety outcome was the proportion of patients who experienced a grade 3 or 4 adverse event (defined by Division of AIDS [DAIDS] 2017 Adverse Event grading criteria) up to 1 month after treatment completion. On-site and remote laboratory monitoring ensured completeness of reporting.

Secondary Outcomes

Secondary outcomes included per protocol analysis of the primary efficacy outcome (PP-M), primary efficacy outcome in the inclusive trial populations (mITT-All; PP-All), time-to-event analyses of the primary efficacy outcome (mITT-M; PP-M); sputum culture conversion status at 8 and 12 weeks from randomization and time to culture conversion (mITT-M; PP-M), and the proportion of participants who experienced an adverse event of any grade (safety).

Subgroup Analysis

We also pre-specified a subgroup analysis of the primary outcome among those with and without cavitation on chest x-ray at baseline; according to baseline sputum smear grade; and according to baseline quantitative Xpert/MTB line probe assay. Subgroup analysis based on quantitative Xpert/MTB cycle threshold (CT) excluded those with the lowest 10% of CT values. This was a pragmatic decision based on the distribution of CT values, with the aim of identifying a subgroup that excluded only a minority of the population. Full details are available in the statistical analysis plan, section 6.

Statistical Analysis

Assuming a proportion of participants with unfavorable outcome in the control regimen of 7%, and that up to 20% of randomized participants might be late exclusions or unassessable because of drug resistance or loss to follow-up, we calculated a sample size of 654 microscopy smear-positive, Xpert MTB/RIF-positive, rifampicin-susceptible patients (218 per arm) would provide a minimum of 525 evaluable participants, giving 90% power to test the hypothesis that SR2 was non-inferior to control, with a non-inferiority margin of 8% and one-sided significance level of 0.05. For the primary outcome, to control the family-wise type I error rate, we employed a fixed sequence of ordered hypotheses. Non-inferiority of SR2 was tested first, with non-inferiority of SR1 formally tested only if SR2 demonstrated non-inferiority to control. As randomization was stratified by trial site, all statistical analyses include adjustment for site. Full details of the sample size parameters and justification of the margin of non-inferiority are described in the statistical analysis plan, sections 3.1 and 3.2.

RESULTS

Trial population

Between January 2017 and December 2020, 672 participants were randomized across trial sites including Uganda (224), Guinea (175), Peru (119), Nepal (70), Botswana (54), and Pakistan (30). Two-hundred twenty-four, 223, and 225 participants were assigned to CR, SR1 and SR2, respectively. All randomized participants received at least one dose of trial medication and the treatment to which they were allocated. Across arms, 12 participants assigned to CR, 11 assigned to SR1, and 13 assigned to SR2 fulfilled the late exclusion criteria; 29 of these 36 were

due to baseline drug resistance (Figure 1). After removal of late exclusions, 212 participants were included in each trial arm for the mITT-All population. Twenty-one participants assigned to CR, 20 assigned to SR1, and 17 assigned to SR2, had no documented positive smear or culture result at baseline and were excluded from the primary mITT-M population. The mITT-M population included 191 participants in CR (including 4 unassessable outcomes), 192 in SR1 (6 unassessable), and 195 in SR2 (9 unassessable) (Figure 1).

Baseline characteristics of participants were similar in the three arms (mITT-M population, Table 1; safety population, Table S2), and are broadly reflective of the global population with tuberculosis, with the notable exception that the trial population excludes participants with HIV and diabetes (Table S7).

Adherence to trial medication was consistent across groups: between 88-90% of participants were recorded as taking all doses of trial medication in each of the trial arms. Among participants who completed the trial with a favorable outcome, adherence was universally excellent, with >98% recorded taking all doses in each trial arm. Consequently, no such participants were excluded from per protocol analysis due to not receiving adequate treatment.

Primary Outcome

Comparing SR2 with the control group, non-inferiority was not demonstrated. In the primary mITT-M population, an unfavorable outcome occurred in 13.4% (n=25) of participants in SR2 and 7.0% (n=13) in the control group, for an adjusted absolute risk difference of 6.3 percentage

points (90% confidence interval: 1.1 to 11.5; P-value for hypothesis test of non-inferiority = 0.295) (Figures 2 and 3, Table 2). According to the fixed sequence of ordered hypotheses, formal non-inferiority testing of SR1 was not performed. An unfavorable outcome occurred 10.2% (n=19) of participants in SR1, for an adjusted absolute risk difference of 3.1 percentage points (-1.6 to 7.9) compared to control. The reasons for unfavorable outcome are shown in Table 2. Eight more participants withdrew during the treatment phase or had a change of treatment due to adverse events in SR2 than in SR1 or control. There were the same number of culture-confirmed relapses in SR2 and SR1, both more than in control.

Secondary Outcomes

Results comparing SR2 and SR1 to control in the secondary analysis populations of mITT-All, and the per protocol populations PP-M and PP-All, were similar to those seen in the primary outcome analysis (Figure 2). The time from randomization to an unfavorable outcome for the primary analysis population (mITT-M) population is shown in Figure 3, and for the PP-M population in Figure S2. Week 8 culture conversion was 158/184 (85.9%), 166/179 (92.7%), and 164/182 (90.1%) for control, SR1, and SR2, respectively. Week 12 culture conversion was 182/185 (98.4%), 180/184 (97.8%), and 184/187 (98.4%) for the control arm, SR1, and SR2, respectively (Table 2).

Subgroup Analysis

Pre-specified subgroup analyses were performed for the primary analysis population (mITT-M). In subgroups excluding those with the most severe disease at baseline, the unfavorable

outcome rates for SR1 were close to those for the control arm. Specifically, excluding those with far advanced disease and cavitation on chest x-ray, an unfavorable outcome occurred in 4.4% (4/91) of participants in SR1 and 4.5% (4/88) in control, giving an adjusted risk difference of -0.3 percentage points (-5.4 to 4.9) (Figure S4). Excluding the lowest 10th percentile on baseline semi-quantitative Xpert cycle threshold (those with highest organism load), an unfavorable outcome occurred in 8.9% (15/169) of participants in SR1 and 7.3% (12/165) in control, giving an adjusted risk difference of 1.6 percentage points (-3.2 to 6.5) (Figure S4). Results for those with far advanced disease on chest x-ray are presented in Figure S5.

Post Hoc Analysis

In a post-hoc analysis of the primary outcome adjusted for site, age, and baseline lung grading on chest x-ray, the comparison of SR2 and control gave an adjusted risk difference of 3.6 percentage points (-1.7 to 9.0); the comparison of SR1 and control gave an adjusted risk difference of 3.0 percentage points (-1.8 to 7.8) (Figure S1).

Safety and adverse events

The proportion of participants experiencing a grade 3 or 4 adverse event was 4.0%, 4.5%, and 4.4% in the control group, SR1, and SR2, respectively (Table 3). There were three serious adverse events (1.3%) in each arm. There were 5 (2.2%) deaths in total in the control group, 8 (3.6%) in SR1, and 3 (1.3%) in SR2. Six post-treatment deaths were determined to be unrelated to TB or TB treatment by the independent endpoint review committee (2 in the control arm, 1 in SR1, and 3 in SR2).There were more cases of a grade 4 ALT rise or a grade 3 or 4 increase in

bilirubin in SR2 than in SR1 or control, although the proportion of participants with such rises was low (Table 3). Additional details of liver injury events appear in Table S5. A complete listing of adverse events leading to a change in allocated trial treatment is presented in Table S4.

DISCUSSION

The trial did not identify a treatment regimen that was non-inferior to control according to our pre-defined criteria. The primary outcome favorable response rates were 93%, 90% and 87% in the control, SR1, and SR2 arms, respectively.

Although comparisons across trials should be made with caution, the response rates were generally higher and risk differences from control generally lower than seen in previously tested 4-month regimens in the RIFAQUIN, REMOX, and OFLOTUB trials[23-25]. Notably, our results (Figure 2 and Figure S1) are similar to the results seen in the 4-month, high dose rifapentine (without moxifloxacin) arm in the recent TBTC Study 31/A5349 trial[19], with the assessable population results for that arm (percent point difference from control 4.6, Figure 2A in reference 19) representing a similar analytical approach to that used in our trial. The data suggest that our trial participants had at least comparable disease severity to those in these other trials: cavitation was seen in 87% of RIFASHORT participants and 73%, 72%, 65%, and 51%, respectively of participants in the TBTC Study 31/A5349, REMOX, RIFAQUIN, and OFLOTUB trials. High-grade (3+) sputum smears were seen in 33% in RIFASHORT and 27% in TBTC Study 31/A5349.

The 1200mg/d rifampicin arm was associated with marginally fewer hepatic adverse events than the 1800mg/d rifampicin arm. However, the rates of adverse events and of hepatic events were less than 5%, and events were reversible, meaning that these data do not destroy the clinical equipoise needed for the continued trial of rifampicin doses of 1800 mg/d and above, as are ongoing in tuberculous meningitis and tuberculosis in advanced HIV disease[26]. The slight increase in hepatic events in the 1800mg/d arm may explain some of the apparent difference in efficacy between doses, with more unfavorable outcomes in the 1800 mg/d arm resulting from treatment change due to adverse events and withdrawal during treatment. In analyses adjusted by factors known to be associated with outcome (site, age, lung grading, gender), the risk difference results were more similar comparing the 1200mg and 1800mg doses, suggesting that some of the underperformance of the 1800mg/d and experimental arms may be explained by small differences in baseline characteristics. In addition, in those patients with advanced disease on chest x-ray, results slightly favored the 1800mg/d over the 1200 mg/d dose (Figure S5).

In the 1200mg/d and 1800mg/d arms, culture-confirmed relapses were the same, and culture conversion was similar at 2 and 3 months. In both experimental arms, culture conversion was higher at 2 months than in control, and culture-confirmed relapse rates were less (4.8% in both 1200 mg and 1800 mg arms) than the 12% seen in historical 4-month regimens using rifampicin at 10 mg/kg[27]. Of note, given a median weight of participants of 52kg, 1800 mg/d was equivalent to 35 mg/kg/d or more for half of all participants, a dosing level associated with more rapid bactericidal activity and culture conversion in prior clinical studies[13,15].

In subgroup analyses excluding the most severe disease, results for the 1200mg/d regimen were close to those for the control arm, including among 90% of the trial population without the lowest Xpert cycle threshold values at baseline. These data support a simple stratified approach to treatment. Cycle threshold is available wherever GeneXpert is used, and could be used to identify the 10% of patients with the highest organism load for 6 months of therapy.

Limitations of our trial include the fact that we did not include participants with HIV infection or diabetes. When the trial was designed, antiretroviral therapy (ART) frequently included efavirenz, and the degree of interaction between efavirenz and high dose rifampicin was uncertain. With dolutegravir-based ART, significant drug interaction is no longer an issue, and planned follow-up studies will include participants with HIV and diabetes to maximize generalizability. In addition, we did not collect individual-level rifampicin pharmacokinetic data. Analyses of the TBTC Study 31/A5349 trial demonstrated the importance of individual-level rifapentine exposure[19]. We used solid media for sputum cultures, available across all trial sites, and, where available, mycobacteria growth indicator tube cultures. Ours was a pragmatic trial across low- and middle-income country settings, and pharmacokinetic assessments and setting up of new mycobacterial growth indicator tube culture facilities was not possible within our resources. Finally, it is possible that some initial nervousness around the higher dose may have prompted earlier treatment changes than would otherwise have occurred in participants on the higher dose, although this only seems to have been a possibility in one case.

In conclusion, 4-month regimens including high-dose rifampicin were associated with few adverse events but did not meet non-inferiority criteria. Efficacy results were closely in line with those of the high dose rifapentine alone arm of TBTC Study 31/A5349. Ongoing studies are planned, incorporating moxifloxicin and simple stratification of treatment duration by cycle threshold.

ACKNOWLEDGEMENTS AND FUNDING

Supported by a grant through the Wellcome Trust / Medical Research Council (UK) / UKAID Joint Global Health Trials (MR/N006127/1). This work was also funded by The Aga Khan Foundation, Canada.

We thank all the trial participants, their families and caregivers, as well as all the clinical, laboratory and administrative staff at all the sites who were not directly involved in the trial; Gerry Davis, Andrew Nunn, and Payam Nahid for serving on the data and safety monitoring committee; and Peter Godfrey-Faussett, Alwin Mwinga, Patrick Phillips, and Andrew Vernon for serving on the trial steering committee.

REFERENCES

1. Global Tuberculosis Report, World Health Organization. 2020.
2. Gomez GB, Dowdy DW, Bastos ML, Zwerling A, Sweeney S, Foster N, Trajman A, Islam MA, Kapiga S, Sinanovic E, Knight GM, White RG, Wells WA, Cobelens FG, Vassall A. Cost and cost-effectiveness of tuberculosis treatment shortening: a model-based analysis. BMC Infect Dis 2016 Dec 1;16(1):726. doi: 10.1186/s12879-016-2064-3.
3. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. Int J Tuberc Lung Dis 2000;4:796–806.
4. van Ingen J, Aarnoutse RE, Donald PR, et al. Why do we use 600 mg of rifampicin in tuberculosis treatment? Clin Infect Dis 2011;52:e194–9.
5. Liu Y, Pertinez H, Ortega-Muro F, Alameda-Martin L, Harrison T, Davies G, Coates A, Hu Y. Optimal doses of rifampicin in the standard drug regimen to shorten tuberculosis treatment duration and reduce relapse by eradicating persistent bacteria. J Antimicrob Chemother. 2018 Mar 1;73(3):724-731. PMID: 29244108
6. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrobial agents and chemotherapy* 2003; 47:2118–24.
7. Rosenthal I, Tasneen R, Peloquin C, et al. Dose-ranging comparison of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis. *Antimicrobial agents and chemotherapy* 2012; 56:4331–40.
8. de Steenwinkel JE, Aarnoutse RE, de Knegt GJ, et al. Optimization of the rifampin dosage to improve the therapeutic efficacy in tuberculosis treatment using a murine model. *American journal of respiratory and critical care medicine* 2013; 187:1127–34.

9. Hu Y, Liu A, Ortega Muro F, Alameda Martin L, Mitchison D, Coates A. High-dose rifampicin kills persisters, shortens treatment duration, and reduces relapse rate in vitro and in vivo. *Frontiers in microbiology* 2015; 6:641.
10. Diacon AH, Patientia RF, Venter A, van Helden PD, Smith PJ, McIlleron H, Maritz JS, Donald PR. Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears. Antimicrob Agents Chemother. 2007 Aug;51(8):2994-6.
11. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. Antimicrob Agents Chemother. 2007; 51(7):2546-51.
12. Steingart KR, Jotblad S, Robsky K, Deck D, Hopewell PC, Huang D, Nahid P. Higher-dose rifampin for the treatment of pulmonary tuberculosis: a systematic review. Int J Tuberc Lung Dis. 2011;15(3):305-16.
13. Boeree MJ, Diacon AH, Dawson R, Narunsky K, du Bois J, Venter A, Phillips PP, Gillespie SH, McHugh TD, Hoelscher M, Heinrich N, Rehal S, van Soolingen D, van Ingen J, Magis-Escurra C, Burger D, Plemper van Balen G, Aarnoutse RE; PanACEA Consortium. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med. 2015;191(9):1058-65.
14. Jindani A, Borgulya G, Westermann de Patino I, Gonzales T, Alvarez de Fernandes R, Shrestha B, Atwine D, Bonnet M, Burgos M, Dubash F, Patel N, Checkley A, Harrison T, Mitchison D. A randomised Phase II trial to evaluate the toxicity of high dose rifampicin to treat pulmonary tuberculosis. *Int J Tuberc Lung Dis.* 2016;20(6):832–838.
15. Boeree MJ, Heinrich N, Aarnoutse R, Diacon AH, Dawson R, Rehal S, et al. PanACEA Consortium. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. *Lancet Infect Dis.* 2017; 17:39–49.

16. Aarnoutse RE, Kibiki GS, Reither K, Semvua HH, Haraka F, Mtabho CM, et al. PanACEA Consortium. Pharmacokinetics, tolerability, and bacteriological response of rifampin administered at 600, 900, and 1,200 milligrams daily in patients with pulmonary tuberculosis. *Antimicrob Agents Chemother.* 2017; 61:e01054–17.
17. Svensson EM, Svensson RJ, Te Brake LHM et al. The potential for treatment shortening with higher rifampicin doses: relating drug exposure to treatment response in patients with pulmonary tuberculosis. *Clin Infect Dis* 2018; 67:34–41.
18. Velásquez GE, Brooks MB, Coit JM, Pertinez H, Vargas Vásquez D, Sánchez Garavito E, Calderón RI, Jiménez J, Tintaya K, Peloquin CA, Osso E, Tierney DB, Seung KJ, Lecca L, Davies GR, Mitnick CD. Efficacy and Safety of High-Dose Rifampin in Pulmonary Tuberculosis. A Randomized Controlled Trial. Am J Respir Crit Care Med. 2018; 198(5):657-666.
19. Dorman SE, Nahid P, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, Engle M, Goldberg SV, Phan HTT, Hakim J, Johnson JL, Lourens M, Martinson NA, Muzanyi G, Narunsky K, Nerette S, Nguyen NV, Pham TH, Pierre S, Purfield AE, Samaneka W, Savic RM, Sanne I, Scott NA, Shenje J, Sizemore E, Vernon A, Waja Z, Weiner M, Swindells S, Chaisson RE; AIDS Clinical Trials Group; Tuberculosis Trials Consortium. Four-Month Rifapentine Regimens with or without Moxifloxacin for Tuberculosis. N Engl J Med. 2021 May 6;384(18):1705-1718.
20. Alfarisi O, Alghamdi WA, Al-Shaer MH, Dooley KE, Peloquin CA. Rifampin vs. rifapentine: what is the preferred rifamycin for tuberculosis? Expert Rev Clin Pharmacol. 2017; 10(10):1027-1036.
21. Nunn AJ, Phillips PP, Mitchison DA. Timing of relapse in short-course chemotherapy trials for tuberculosis. Int J Tuberc Lung Dis. 2010 Feb;14(2):241-2.
22. Witney AA, Bateson AL, Jindani A, Phillips P, Coleman D, Stoker NG, Butcher PD, McHugh TD, and RIFAQUIN Study Team. Use of whole genome sequencing to distinguish relapse from reinfection in a completed tuberculosis clinical trial. BMC Med. 2017; 15:71.

23. Jindani A, Harrison TS, Nunn A, Phillips P, Churchyard G, Charalambous S, Hatherill M, Geldenhuys H, McIlleron H, Zvada S, Mungofa S, Syed N, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk J, Clouting H, Coleman D, Bateson A, McHugh T, Butcher P, Mitchison D, on behalf of the RIFAQUIN trial team. RIFAQUIN: A multicentre randomised controlled clinical trial to evaluate high dose rifapentine and a quinolone in the treatment of pulmonary tuberculosis. New England J Med 2014; 371:1599-1608.
24. Merle CS, Sismanidis C, Sow OB, et al. A pivotal registration phase III, multicenter, randomized tuberculosis controlled trial: design issues and lessons learnt from the Gatifloxacin for TB (OFLOTUB) project. Trials 2012; 13: 61.
25. Gillespie SH, Crook AM, McHugh TD et al. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med 2014; 371:1577–87
26. https://www.isrctn.com/ISRCTN15668391; https://clinicaltrials.gov/ct2/show/NCT04951986; https://clinicaltrials.gov/ct2/show/NCT04145258?id=NCT04145258&draw=2&rank=1
27. Fox W. Whither short course chemotherapy? Br J Dis Chest 1981; 75:331-357.

Figure Legends

Figure 1. Screening, randomization, and analysis populations (CONSORT). Participants may have had more than one reason for exclusion. MTB denotes *Mycobacterium tuberculosis*, H isoniazid, R rifampicin, Z pyrazinamide, E ethambutol, mITT modified intention to treat.

Figure 2. Differences from the Control Regimen in Unfavorable Outcome Rates (90% Confidence Intervals). Closed square: primary outcome analyses; Closed triangle: secondary outcome analyses. The dashed line indicates the pre-specified 8% non-inferiority margin. aRD denotes adjusted risk difference, mITT modified intention to treat, PP per protocol. Formal testing of the non-inferiority hypothesis for SR2 in the mITT-M population yields a p-value of 0.295.

Figure 3. Kaplan Meier Time to unfavorable outcome for the primary mITT-M population**.** The inset shows the same data on an enlarged y axis. aHR denotes adjusted hazard ratio.

TABLES

Table 1. Baseline characteristics of the participants (mITT-M population)

Characteristic	Control (N=191)	Study regimen 1 (N=192)	Study regimen 2 (N=195)
Age - n (%)			
Median (IQR)	29.0 (23.0 - 38.0)	29.0 (22.0 - 36.0)	28.0 (23.0 - 43.0)
18-24	57 (29.8)	64 (33.3)	72 (36.9)
25-34	73 (38.2)	69 (35.9)	53 (27.2)
>34	61 (31.9)	59 (30.7)	70 (35.9)
Weight - n (%)			
Median (IQR)	52.2 (47.0 - 57.7)	51.9 (46.8 - 58.1)	52.6 (48.0 - 58.0)
BMI			
Median (IQR)	18.4 (16.9 - 20.2)	18.6 (16.9 - 20.8)	18.8 (17.0 - 21.0)
Sex - n (%)			
Female	54 (28.3)	41 (21.4)	49 (25.1)
Ethnicity - n (%)			
African	136 (71.2)	133 (69.3)	134 (68.7)
Hispanic	1 (0.5)	2 (1.0)	1 (0.5)
Mixed	32 (16.8)	34 (17.7)	36 (18.5)
Indigenous (South American)	1 (0.5)	1 (0.5)	0 (0.0)
Asian	21 (11.0)	22 (11.5)	24 (12.3)
Smoking status - n (%)			
Current	47 (24.6)	33 (17.2)	36 (18.5)
Former	15 (7.9)	15 (7.8)	17 (8.7)
Never	129 (67.5)	144 (75.0)	142 (72.8)
CXR cavitation - n (%)			
Unreadable/unknown	0	1 (0.5)	1 (0.5)
Yes	165 (86.4)	174 (90.6)	174 (89.2)
No	26 (13.6)	17 (8.9)	20 (10.3)
CXR grading - n (%)			
Unreadable/unknown	3 (1.6)	1 (0.5)	3 (1.5)

Characteristic	Control (N=191)	Study regimen 1 (N=192)	Study regimen 2 (N=195)
Normal or minimal disease	3 (1.6)	4 (2.1)	4 (2.1)
Moderately advanced disease	86 (45.0)	88 (45.8)	85 (43.6)
Far advanced disease	99 (51.8)	99 (51.6)	103 (52.8)
Sputum smear grading - n (%)			
+ or scanty	68 (35.6)	77 (40.1)	81 (41.5)
++	52 (27.2)	41 (21.4)	49 (25.1)
+++	71 (37.2)	74 (38.5)	65 (33.3)

BMI: Body mass index; CXR: Chest X-ray; IQR: Interquartile range.

Table 2. Primary and Key Secondary Outcome Analyses

mITT-M primary analysis assessable outcomes	Control (N=187)	Study regimen 1 (N=186)	Study regimen 2 (N=186)
Favorable			
Participants with outcome - n (%)	174 (93.0)	167 (89.8)	161 (86.6)
Unfavorable			
Participants with outcome - n (%)	13 (7.0)	19 (10.2)	25 (13.4)
Adjusted risk difference to control (95% CI)		3.1 (-1.6 to 7.9)	6.3 (1.1 to 11.5)
Death during the treatment phase	3 (1.6)	4 (2.2)	0
Post-treatment death, TB a plausible cause	0	1 (0.5)	0
Lost to follow-up during the treatment phase	2 (1.1)	0	1 (0.5)
Withdrew from the trial during the treatment phase[1]	3 (1.6)	2 (1.1)	5 (2.7)
Change in treatment due to adverse event[2]	1 (0.5)	2 (1.1)	7 (3.8)
Two consecutive positive cultures after completing treatment	2 (1.1)	9 (4.8)	9 (4.8)
Retreated for TB due to clinical signs and symptoms without two consecutive positive cultures	2 (1.1)	1 (0.5)	3 (1.6)
Unassessable Outcomes	4	6	9
Post-treatment death deemed unrelated to TB or treatment	2	1	2
Post-treatment LTFU when culture negative	1	3	5
Evidence of exogenous TB reinfection	0	1	2
Withdrawal during the treatment phase when culture negative	1	0	0
Post-treatment withdrawal when culture negative	0	1	0
Secondary analysis outcomes			
Confirmed culture conversion from positive to negative – n/N (%)			

mITT-M primary analysis assessable outcomes	Control (N=187)	Study regimen 1 (N=186)	Study regimen 2 (N=186)
Eight weeks from randomization	158/184 (85.9)	166/179 (92.7)	164/182 (90.1)
Twelve weeks from randomization	182/185 (98.4)	180/184 (97.8)	184/187 (98.4)

[1]Reasons for withdrawal during treatment phase: Control: 3 moved home address; SR1: 1 unhappy with the allocated regimen, 1 moved home address; SR2: 2 concerned about high dose treatment, 1 unhappy with the allocated regimen, 1 concerned about high dose treatment exacerbating a pre-existing medical condition, 1 moved home address.
[2]All changes in treatment due to AE involved high liver transaminases or jaundice, except for one in SR1 due to depression. These events are described in supplementary Table S4.
TB denotes tuberculosis.
LTFU denotes lost to follow up.

Table 3. Laboratory-defined and Clinical Adverse Events according to treatment arm

Participants experiencing	Control (N=224)	Study regimen 1 (N=223)	Study regimen 2 (N=225)
Primary safety outcome			
Grade 3 or 4 adverse event - n (%)	9 (4.0)	10 (4.5)	10 (4.4)
Percentage-point difference from control (95% CI)		0.5 (-3.3 to 4.2)	0.4 (-3.3 to 4.2)
Secondary safety outcome			
Grade 1 to 4 adverse event - n (%)	120 (53.6)	109 (48.9)	115 (51.1)
Percentage-point difference from control (95% CI)		-4.7 (-13.9 to 4.6)	-2.5 (-11.7 to 6.8)
Other safety outcomes			
Serious adverse event - n (%)	3 (1.3)	3 (1.3)	3 (1.3)
Notifiable adverse event - n (%)	10 (4.5)	13 (5.8)	13 (5.8)
Notifiable adverse event, excluding pregnancy - n (%)	6 (2.7)	11 (4.9)	13 (5.8)
Death - n (%)	5 (2.2)	8 (3.6)	3 (1.3)
Hepatotoxicity outcomes			
ALT > 180 U/L (5xULN - grade 3) - n (%)	3 (1.3)	7 (3.1)	7 (3.1)
ALT > 360 U/L (10xULN - grade 4) - n (%)	2 (0.9)	1 (0.4)	4 (1.8)
Grade 3/4 ALT results, U/L - median (IQR; max)	387 (237 to 511; 511)	212 (189 to 350; 449)	377 (332 to 450; 942)
Total bilirubin > 3 mg/dL (2.6xULN - grade 3) - n (%)	1 (0.4)	1 (0.4)	6 (2.7)
Total bilirubin > 6 mg/dL (5xULN - grade 4) - n (%)	1 (0.4)	0	3 (1.3)
Grade 3/4 total bilirubin results, mg/dL - median (IQR; max)	12.1	3.2	5.4 (4.1 to 9.4; 29.5)
Satisfies Hy's law (ALT > 3xULN and total bilirubin > 2xULN) - n (%)	0	1 (0.4)	2* (0.9)

* 2 additional participants met the ALT and total bilirubin criteria for Hy's law, however, both were hepatitis B surface antigen (HBsAg) positive, meaning they do not satisfy Hy's law. These events are described in supplementary Table S5. ALT denotes alanine aminotransferase; ULN: upper limit of normal; IQR: Interquartile range; Max: Maximum.