Reviewer's report

Title: Randomized Pharmacokinetic Evaluation of Different Rifabutin Doses in African HIV-infected Tuberculosis Patients on Lopinavir/Ritonavir-based Antiretroviral Therapy

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Reviewer: Kelly Dooley

Reviewer's report:

This is a pretty well-written, timely article that addresses co-treatment of HIV and tuberculosis among those patients who require boosted protease inhibitors for treatment of their HIV and a rifamycin-based TB regimen. Previous guidance documents have recommended a dose of rifabutin of 150 mg thrice-weekly when rifabutin is given with a boosted protease inhibitor, but more recent data suggest that this dose adjustment (made because ritonavir is an inhibitor of cytochrome P450 3A) results in rifabutin concentrations that are subtherapeutic, which could lead to rifamycin drug resistance (the worst possible treatment outcome for HIV-TB co-infected patients). This article provides further evidence that daily rifabutin rather than thrice-weekly rifabutin is appropriate, and will serve to inform and shore up guidelines for co-treatment. I have some suggestions that I think will strengthen this article, which I hope the journal will choose to publish:

(1) Abstract: In the background section, state what the recommended dose is and describe the risk of acquired drug resistance with suboptimal dosing (perhaps move the statement about target AUC of 4.5 mcg*h/mL to the background section - it is not a result). Also why you care about and are measuring the metabolite. MINOR ESSENTIAL

(2) Abstract: Restate the first sentence in the methods section to make it more clear what was done, something along the lines of, "Patients with TB/HIV co-infection received isoniazid, pyrazinamide, and ethambutol at standard doses plus rifabutin 300 mg QD, then were randomized to receive RBT 150 mg thrice-weekly with LPV/r-based ART followed by RBT 150 mg daily with LPV/r-based ART or to these same regimens but in reverse order." Each RBT dosing period lasted 4 weeks. MINOR ESSENTIAL

(3) Abstract: The number of participants in the trial must be reported. MINOR ESSENTIAL

(4) Abstract: Please provide results of the AUC and Cmax comparisons (like GMR with 90% CI) rather than just saying there was a significant reduction. The reader wants to see the magnitude of the reduction. Also, please report the magnitude of increase in 25-O-desacetylrifabutin- this is quite important and may be of clinical concern. MAJOR
(5) Abstract: State the grade of the uveitis and that the neutropenia was asymptomatic. MINOR ESSENTIAL

(6) Background: Is the effect of rifampin on protease inhibitors simply on bioavailability? My impression is that clearance is impacted as well. Please clarify.

(7) Background: The metabolic pathway for rifabutin and for its desacetyl-metabolite should be described here. In addition, the PK parameter most closely associated with treatment outcomes for RBT and the target value of that PK parameter that prevents resistance should be stated. Concerns related to high metabolite concentrations should be outlined here. In addition, many guidelines now recommend daily RBT with boosted PIs, not thrice-weekly RBT (CDC, US DHHS http://aidsinfo.nih.gov/guidelines/html/4/adult-and-adolescent-oi-prevention-and-treatment-guidelines). The authors could state that different guidelines recommend different dosing and provide citations to show that guidelines differ and point out that these differences likely exist because the data are limited, so this study provides data to inform these guidelines. MAJOR

(8) Methods: Was written informed consent provided by each participant? Was the trial registered on a trials website? If so, please state explicitly.

(9) Methods: Why was the study population limited to those with CD4 counts of 50 to 200 cels/mm3?

(10) Methods: The study population includes patients who "had not received ART therapy in the preceding three months". Were most participants treatment-naive, or were they individuals who could not receive EFV-based ART for some reason? Did the investigators feel that the experimental treatment regimens were likely to be of similar efficacy to EFV-based ART with rifampin-containing TB treatment? That is, was there equipoise about this? May consider describing this somewhere in the article.

(11) Methods: Please add a figure showing the study Schema. It is hard to follow just reading the Treatments under study section. The reader will understand the treatments provided and the randomization more readily with a figure to provide a visual representation of study design. MAJOR

(12) Methods: Please state when the safety visits were conducted explicitly rather than saying they were done at PK visits and at the penultimate visit. MINOR ESSENTIAL

(13) Methods: Begin the sample size paragraph with the second sentence, and include how the coefficient of variance was incorporated into sample size calculations. Then describe why this number of 12 was inflated to 16 to account for possible drop-outs or inevaluable PK samples. MINOR ESSENTIAL

(14) Methods: Please provide a reference for the rifabutin/metabolite validated assay, if available.
(15) Methods: Please provide both intra-day and inter-day CVs for both assays (that is, make reporting of accuracy and CV for RBT and LPV consistent).

(17) Methods: "As there was no significant effect of sequence, the two arms were pooled." Effect of sequence on what? "model was used to test the dose effect after adjusting for baseline differences". Dose effect on what?

(18) Methods: "and the 150 mg TPW at 48 hours with 2 x baseline at 24 hours because the dosing interval is 48 hours for hte TPW dose" could be modified to be clearer, possibly "the AUC0-48 was compared for 150 mg TWP and 300 mg QD. To determine the AUC0-48 for the 300 mg QD dose, the value for the AUC0-24 was simply doubled."

(19) Results: Please provide the dates during which the study was conducted, either in the methods or the results. MINOR ESSENTIAL

(20) Results: "Two participants were prematurely withdrawn from the study and thus were not evaluable for PK analyses" rather than "two participants were withdrawn from the pharmacokinetic analysis."

(21) Results: Please describe the GMR with 90% CI of key PK parameters (Cmax, AUC) briefly in the text rather than providing p-values. Reader is more interested in the magnitude of the interaction, not whether or not it is statistically significant. A highly statistically-significant interaction can be of modest magnitude and have little clinical relevance. MAJOR

(22) Results: Figure 1 is not referenced in the results section. Please correct this. MINOR ESSENTIAL

(23) Results: A linear mixed model is described in the methods, but the model results are not provided in the results. Please either remove the description of the methods or provide the results. MINOR ESSENTIAL

(24) Results: "considered by the study team to be unrelated to rifabutin" rather than "unrelated to rifabutin".

(25) Results: The dose of RBT that the patients were taking, and the timing of the AEs in the treatment course should be stated in the Adverse Events paragraph for all the important AEs--uveitis, neutropenia, and transaminitis. This is really important because the reader is wondering if all these events happened while patients were getting RBT 150 mg daily with LPV/r. I would suggest adding a small table that describes these 3 AEs (uveitis, hepatitis, neutropenia--the most relevant ones for rifabutin)--grade, treatment at the time of the AE, week on study at the time of the AE, AUC where available. MAJOR

(26) Discussion: This is really a very boring summary first paragraph despite the results of this study being quite interesting. Would spice up this first paragraph of the discussion by stating that daily rather than thrice-weekly seems to achieve targets that reduce risk of resistance but that metabolite exposures went way up.
These results support use of once-daily rather than thrice-weekly dose of RBT.

(27) Discussion: There is no description of the limitations of this study. Please add a paragraph to describe the limitations, as, in my view, every paper should have a section describing strengths and limitations. Lack of a standard treatment group or large numbers to understand risk of higher exposures to metabolite would be one. Investigators are best-situated to provide these. Strengths would include evaluation of all three RBT dosing regimens in each participant, allowing for intra-patient comparisons and evaluation in patients with TB/HIV co-infection.

(28) Discussion: Please update the references to include studies comparing boosted PI with RBT daily vs. RBT thrice weekly. Lan et al PLoS One 2014 9:e84866, for example.

(29) Discussion: Please describe why you think the fact that the formulation of the drug in this study is Aluvia is important. This is stated as new in abstract and discussion, but it's not clear to me why this really matters.

(30) Discussion: The authors describe a reduction in bioavailability in the third paragraph of the discussion, but bioavailability was not measured or compared.

(31) Discussion: The fact that 29% of patients had an AUC less than 4.5 while taking RBT daily versus 86% taking RBT thrice-weekly is buried deep in the discussion. Consider highlighting this more strongly, either by adding it to the results or by putting it into the conclusions paragraph.

(32) Discussion: Provide evidence for the statement that low Cmin values for rifabutin or rifamycins in general may select for resistance. In some places in the manuscript, target PK parameter is unknown, in some places it is AUC, and now Cmin may be important. Please review statements about PK correlates about efficacy/prevention of resistance for consistency.

(33) Discussion: Does the word "therapeutic index" apply to metabolites? or just to the administered drug?

(34) Discussion/Conclusions: What does the following statement mean: "There has been a reluctance to recommend a higher dose of rifabutin partly based on early experience with rifabutin and CYP3A4 inhibitors?" Please clarify what the concern is that results in this reluctance. From the references, it appears that the concern is that rifabutin may have a narrow therapeutic margin and higher parent or metabolite concentrations may result in higher risk of uveitis or neutropenia. Please describe these concerns in more detail, including the pediatric study by McIlreron and colleagues that had to be stopped early because of severe neutropenia. In my view, we don't really know if the 15-fold increase in desacetyl-RBT will be a safety risk, and a 14-patient study (even a well-conducted one like this one) does not give us a lot of safety information. My recommendation would be to strengthen the language in the conclusions about
safety. While parent drug concentrations are more commonly in therapeutic range with 150 mg daily and risk of resistance likely reduced, metabolite concentrations are 15-fold higher and the safety risks are unknown. MAJOR

(35) Conclusions: The statement that neutropenia risk with rifabutin will be reduced by avoiding cotrimoxazole is not supported by any evidence. My suggestion is to remove this sentence, as it can be misleading (particularly to the reader who just reads the abstract and conclusions). MAJOR

(36) Table 2: Please add Cmax and Cmin GMR, as these are referenced in the text. Also, AUC0-48 is the comparison for 150 TPW with ART vs. 300 QD without ART; AUC0-24 is the comparison for 150 QD with ART vs. 300 QD without ART. Please remove the other comparisons. MINOR ESSENTIAL

(37) Figure 1: Consider putting in the "next dose" in the graphs. That is, for those individuals taking daily RBT, at 24 hours, a second dose would be given. For those taking thrice-weekly RBT, no dose would be given at 24 hours. Showing expected concentration-time curves over 48 hours would show very dramatically how seriously low the thrice-weekly dose concentrations are and how really high the once-daily 150 dose is (both compared to 300 mg without ART).

(38) Figure 1. Both lines look dashed rather than one being dotted and the other dashed. Please fix this. MINOR ESSENTIAL

(39) Figure 3. Consider deleting this figure. These data are provided in Table 3, and the figure does not add much interesting information.

(40) Small grammatical or typographic errors: not tolerance but tolerability; in methods "safety of rifabutin and lopinavir" should be "boosted lopinavir" or "lopinavir/ritonavir"; diluted reanalyzed should be "diluted and reanalyed"; "table 3 in figure 3" should be "table 3 and figure 3".

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

No competing financial interests.