PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

(This paper received three reviews from its previous journal but only two reviewers agreed to published their review.)

ARTICLE DETAILS

TITLE (PROVISIONAL)	Evaluation of Metformin in combination with Rifampicin containing Anti-tuberculosis therapy in patients with new, smear-positive pulmonary tuberculosis (METRIF): Study protocol for a randomized clinical trial
AUTHORS	Padmapriyadarsini, Chandrasekaran; P.K., Bhavani; Natrajan, Mohan; C, Ponnuraja; Kumar, Hemanth; Gomathy, N.S.; Guleria, Randeep; M.S., Jawahar; SIngh, Manjula; Tanjore, Balganesh; Swaminathan, Soumya

VERSION 1 – REVIEW

REVIEWER	Dr Kingsley Nnanna Ukwaja Department of Medicine, Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria
REVIEW RETURNED	09-Aug-2018

GENERAL COMMENTS	I have read the study protocol and I believe that the protocol has an excellent design, and all aspects of the Methods Section of the protocol are well-described. Therefore, I have no objection to its publication following review

REVIEWER	Takashi Sato Yokohama City University Graduate School of Medicine, Department of Pulmonology, Japan
REVIEW RETURNED	19-Aug-2018

GENERAL COMMENTS	This study protocol described by Dr. Padmapriyadarsini and colleagues plans based on the recent findings of a novel strategy for host-directed therapy of pulmonary tuberculosis (PTB) by using metformin (MET) in addition to the conventional anti-tuberculosis treatment. The general ideas are promising, and the purpose/methods are clearly mentioned. Here are some comments that may be useful for the authors. Comments: This study protocol described by Dr. Padmapriyadarsini and colleagues plans based on the recent findings of a novel strategy for host-directed therapy of pulmonary tuberculosis (PTB) by using metformin (MET) in addition to the conventional anti-tuberculosis treatment. The general ideas are promising, and the

	purpose/methods are clearly mentioned. Here are some comments that may be useful for the authors. 1) P.6, L.10: Does AIIMS need to be abbreviated because AIIMS does not appear in subsequent manuscript? 2) P.6, L.11: In Study Design and oversight section, “National AIDS Research Institute (NARI)” was already used on P.6, L.7. Abbreviations should be defined upon first mention in text. 3) P.8, L.5: Please specify the reason why authors choose 2HRZE/4HRE regimen as a standard regimen since The World Health Organization recommends the 2HRZE/4HR regimen for new patients with PTB. Reviewer assume that the high prevalence of INH resistance and/or unavailable for INH susceptibility test in the study areas. Please clarify the reason. 4) P.8, L.6: It would be better to specify the reason why authors set the dose of 1000 mg once daily MET in test regimen. Reviewer assume that the dose has been optimized based on the findings from animal study conducted by Singhal et al. (Sci Transl Med 2014;6:263ra159). Especially, in chronic model of mycobacterium tuberculosis infection in mice, Singhal showed that those received combination therapy with 10 mg/kg of INH and 250 mg/kg of MET could significantly decrease the lung bacillary load compared to those treated with only 10 mg/kg of INH. This dose would be equivalent to a MET dose setting in test regimen. Please cite relevant articles regarding the description of “Metformin will be dosed as 500 mg once daily for the 1st week and then 1000 mg once daily for the remaining period of 7 weeks”. 5) P.8, Table 2: Regarding inclusion criteria, subjects having body weight less than 30 kg or over 65 kg would be excluded, thus the table column of “Weight Band” should be modified. 6) P.9, L.15: Is “solid culture” necessary for this study protocol as the study outcome will be analyzed by using MGIT system? 7) P.11, L.8: In Treatment delivery, compliance and retention section, “directly observed therapy (DOT)” was already used on P.8, L.6. Please define abbreviations upon first mention in text. 8) P.12, L.16: In Study Outcome section, “pharmacokinetic (PK)” was already used in Introduction section (P.5, L.17). 9) P.16, L.6: Please spell out the abbreviation “CRFs” if needed, though CRF did not appear in subsequent manuscript. 10) P.18, L.7: In Discussion section,“AMP-activated protein kinase (AMPK)” was already defined on P.5, L.1. 11) P.18, L.9: Regarding the description of “Metformin also acts through AMPK-independent mechanisms. It promotes phagocytosis, phago-lysosome fusion & autophagy in macrophages.”, previous study showed that MET-induced autophagy did not affect bacterial viability (Singhal et al. Sci Transl Med 2014;6:263ra159, Restrepo BI. Tuberculosis 2016;101:S69). Instead, MET-derived mitochondrial ROS would be another key bactericidal mechanism (Singhal et al. Sci Transl Med 2014;6:263ra159, Restrepo BI. Tuberculosis 2016;101:S69). Thus,

	the author may be considered to describe that the potent beneficial mechanisms using MET would be required for further study. 12) P.19, L.3: "time-to-detection (TTD)" was already defined on P.5, L.13.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 2

1) P.6, L.10: Does AIIMS need to be abbreviated because AIIMS does not appear in the subsequent manuscript?

Response: We agree with the reviewer. As this comes only once in the manuscript and not repeated anywhere else, we do not have to abbreviate AIIMS. This has been removed now from P6, L.10.

2) P.6, L.11: In Study Design and oversight section, "National AIDS Research Institute (NARI)" was already used on P.6, L.7. Abbreviations should be defined upon the first mention in the text.

Response: We agree with the reviewer and thank them for pointing this out. We have corrected this now with abbreviations expanded at the first instance.

3) P.8, L.5: Please specify the reason why authors choose 2HRZE/4HRE regimen as a standard regimen since The World Health Organization recommends the 2HRZE/4HR regimen for new patients with PTB. Reviewer assumes that the high prevalence of INH resistance and unavailable for INH susceptibility test in the study areas. Please clarify the reason.

Response: As the reviewer has rightly pointed, due to the high prevalence of Isoniazid mono- or poly resistance, the Revised National TB control programme of India advocates using three drugs in the continuation phase of the anti-tuberculosis treatment namely isoniazid, rifampicin, and ethambutol.

4) **P.8, L.6: It would be better to specify the reason why authors set the dose of 1000 mg once daily MET in test regimen.**

Response: It has been shown in animal model experiments that mice treated with metformin (500 mg/kg) had reduced bacillary load in both lung and spleen, and this dose was equivalent to a metformin dose of 2430 mg/day for a 60-kg human (http://www.naturalhealthresearch. org/extrapolation-of-animal-dose-to-human/) [1]. Such high treatment has also been used in clinical practice for management of non-diabetic conditions like polycystic ovarian syndrome (treatment: 500mg thrice daily to 1g twice daily) [2] and obesity (dose: 750 – 1700mg/day) [3] etc. In this study, we propose to investigate the immune-mediated effect of metformin on TB patients, the time to the intracellular killing of mycobacterium, the drug-drug interaction between metformin and other anti-TB drugs like rifampicin, isoniazid, pyrazinamide, and ethambutol (by measuring their plasma levels). Considering all the facts mentioned above, it was decided to use a higher dose of metformin in this study. We will not be getting treatment naïve TB patients at body weight 60kgs in this setting. Also, there is still not clear evidence about hypoglycemia induced by metformin and rifampicin given together, it was decided not to use very high dosage but to restrict the dose to 1000mg daily for a period of 7-weeks, after the initial 1-week of

titration dose of 500mg (this was done to reduce the gastritis effect of metformin as suggested by experienced diabetologist).

Ref 1: Reigner BG, Blesch KS. Estimating the starting dose for entry into humans: Principles and practice. Eur J Clin Pharmacol 2002; 57: 835-845

Ref 2: Lord JM, Flight IHK, Norman RJ. Metformin in polycystic ovary syndrome: Systematic review and meta-analysis. Br Med J 2003; 327: 951

Ref 3: Levri KM, Slaymaker E, last A et al. Metformin as a treatment for overweight and obese adults. Ann Fam Med 2005; 3 (5): 457-461

5) P.8, Table 2: Regarding inclusion criteria, subjects having a body weight of less than 30 kg or over 65 kg would be excluded. Thus the table column of "Weight Band" should be modified.

Response: We have modified as suggested in P.8, Table 2

6) P.9, L.15: Is "solid culture" necessary for this study protocol as the study outcome will be analyzed by using the MGIT system?

Response: Though the primary study outcome will be analyzed using MGIT system, we also plan to estimate the log reduction in the colony count of mycobacterium tubercle bacilli between the two study arms and will require solid culture for this. We will also be comparing the concordance/discordance between liquid and solid culture results week-wise as an exploratory outcome.

7) **P.11, L.8: In Treatment delivery, compliance and retention section, "directly observed therapy (DOT)" was already used on P.8, L.6. Please define abbreviations upon the first mention in the text**.

Response: We have corrected this now.

8) P.12, L.16: In Study Outcome section, "pharmacokinetic (PK)" was already used in the Introduction section (P.5, L.17).

Response: We thank the reviewer for pointing this out. Abbreviation for Pharmacokinetic as PK has now been added in P5, L17 and removed from P12, L16.

9) P.16, L.6: Please spell out the abbreviation "CRFs" if needed, though CRF did not appear in the subsequent manuscript.

Response: CRF is also used in the section on "Data collection, Management, and Interim analysis." It is expanded now at its first usage and abbreviated with subsequent usage.

10) P.18, L.7: In the Discussion section,"AMP-activated protein kinase (AMPK)" was already defined on P.5, L.1.

Response: This has been corrected now.

VERSION 2 – REVIEW

REVIEWER	Takashi Sato Department of Pulmonology, Yokohama City University Graduate School of Medicine, Japan
REVIEW RETURNED	14-Oct-2018

GENERAL COMMENTS	Comments: The authors have considered all the questions, comments and suggestions, and thus the revised manuscript has been substantially improved. However, reviewer still considers that their response to reviewer 2, comment 3) and 4) should be included in the manuscript (in discussion section) as these concerns would be critical for readers to understand the background dose/regimen optimized. As for reviewer 2, comment 4), please consider again that more recent article by Singhal et al. (Sci Transl Med 2014;6:263ra159) should be cited because they tested 250 mg/kg of MET in combination with 10 mg/kg of INH. This dose of MET would be equivalent to the MET dose planned in this regimen because the dose of 500 mg/kg of MET tested by Reigner BG, et al. (Eur J Clin Pharmacol 2002;57:835) would be equivalent to 2430 mg for a 60-kg human as described in discussion section (P.19, L.9-11).

VERSION 2 – AUTHOR RESPONSE

(i) Reviewer 2 still considers that their comment (3) and (4) should be included in the manuscript (in the discussion section) as these concerns would be critical for readers to understand the background dose/regimen optimized.

(Previous comment 3: Please specify the reason why authors choose 2HRZE/4HRE regimen as a standard regimen since The World Health Organization recommends the 2HRZE/4HR regimen for new patients with PTB. Comment 4: It would be better to specify the reason why authors set the dose of 1000 mg once daily MET in test regimen)

Response: Though the WHO recommends the 2HRZE/4HR regimen for new patients with PTB, given the high prevalence of Isoniazid mono- or poly resistance (11% in new sputum positive patients), the Revised National TB control programme of India recommends using three drugs in the continuation phase of the anti-tuberculosis treatment namely isoniazid, rifampicin, and ethambutol. That is why we are using this regimen and this has now been explained in the Discussion section, page 9, lines 13-16.

(ii) As for reviewer 2, comment (4), please consider again that more recent article by Singhal et al. (Sci Transl Med 2014;6:263ra159) should be cited because they tested 250 mg/kg of MET in combination with 10 mg/kg of INH. This dose of MET would be equivalent to the MET dose planned in this regimen because the dose of 500 mg/kg of MET tested by Reigner BG, et al. (Eur J Clin Pharmacol 2002;57:835) would be equivalent to 2430 mg for a 60-kg human as described in discussion section (P.19, L.9-11).

Response: We agree with the reviewer on this point and have now made this change in the discussion section (p.19, L.9-13. the Corresponding reference also has been updated)