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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

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Abstract

Introduction: Shorter duration of treatment for the management of drug-susceptible pulmonary tuberculosis would be a major improvement in the care of patients suffering from tuberculosis (TB). Besides newer drugs and regimens, other modalities like host-directed therapy are also being suggested to reach this goal. This study's objective is to assess the efficacy and safety of a metformin containing anti-tuberculosis treatment (ATT) regimen in comparison to the standard 6-month ATT regimen in the treatment of newly diagnosed sputum smear positive drug-sensitive pulmonary tuberculosis patients.

Methods and Analysis: We are conducting a multicentric, randomized open-label controlled clinical trial to achieve the study objective. The intervention group will receive isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) along with 1000mg of daily metformin (Met) for the first 2 months while the control group will receive only HRZE. After 2-months, both the groups will receive HRE daily for 4 months. The primary endpoint is time to sputum culture conversion. Secondary endpoints will include time to detection of *mycobacterium tuberculosis* in sputum, pharmacokinetics and pharmacogenomics of study drugs, drug-drug interactions, safety and tolerability of the various combinations and measurement of autophagy and immune responses in the study participants.

Ethics and Dissemination: Study has been approved by the Ethics committee of the participating institutes. Results from this trial will contribute to evidence towards constructing a shorter, effective and safe regimen for TB patients. The results will be shared widely with the National Programme managers, policy makers and stake holders through open access publications, dissemination meetings, conference abstracts and policy briefs. This is expected to

provide a new standard of care for drug-sensitive pulmonary TB patients who will not only reduce the number of clinic visits and lost to follow-up of patients from treatment but also reduce the burden on the health care system.

Trial Registration: The protocol has been registered on Clinical Trial Registry of India with identifier CTRI/2018/01/011176 on January 8th, 2018. This is available on http://ctri.nic.in/Clinicaltrials/rmaindet.php?trialid=20868&EncHid=72267.74662&modid=1&compid=19

Strength and Limitations:

- This controlled clinical trial will add strength to observations from other retrospective and case-control studies that showed metformin to be a protective agent against TB infection among diabetics
- Results from this trial will contribute to evidence towards constructing a shorter, effective and safe regimen for TB patients
- Due to the strict exclusion and inclusion criteria of the clinical trial, the results from the clinical trial may not be generalizable to all groups of TB patients.
- Some degree of heterogeneity of data is expected as it is a multicentric study
- Relapse rates may not be known in this trial as the study duration only for 6 months of treatment in lieu with the study objective

Keywords: Metformin, Pulmonary Tuberculosis, Clinical trial, Short-course therapy, TB treatment

Introduction:

Globally in 2016, there were an estimated 10.4 million new cases of tuberculosis (TB) with five countries, India, Indonesia, China, Philippines and Pakistan accounting for 56% of the total cases [1]. There were an estimated 1.3 million TB deaths in 2016 among HIV-negative people, and an additional 374000 deaths among people living with HIV [1]. Though effective regimens are available for the treatment of drug-sensitive TB with more than 95% cure, the long duration of such regimens have posed problems for TB treatment and control. This, along with drug toxicity results in poor adherence to treatment resulting in emergence of drug resistance.

The global need for new effective therapies has led to a resurgence in efforts to identify additional anti-*M.tb* agents, several of which are now being evaluated in clinical trials. However, conventional pathogen-targeted strategies suffer from the serious disadvantage of fostering microbial resistance. To circumvent this problem, a new paradigm in drug discovery has emerged that involves therapeutic modulation of the host immune responses which leads to better and faster pathogen elimination [2, 3].

An effective host immune system is a crucial factor for both the control and containment of *M.tb* growth. The success of *M.tb* in infecting the host cells and maintaining long-term persistent infection is associated with the ability of bacilli to evade host innate as well as adaptive immune responses [4, 5]. "Host-targeted" adjunct therapeutic strategies not only augment protective host immune responses but also reduce the chance of development of microbial resistance.

One among the host cell innate antimicrobial arsenal includes the capacity to destroy intracellular pathogens using the phagosomal machinery or autophagy pathway. Autophagy is required for the effective control of intracellular pathogens [6] and is regulated by adenosine

monophosphate–activated protein kinase (AMPK) [7]. Accordingly, perturbations in the autophagy network and AMPK signaling have been associated with *M.tb* virulence [8]. The antidiabetic drug Metformin (MET; 1,1-dimethylbiguanide), is an AMPK modulator that inhibits the intracellular growth of *M.tb*, restricts disease immunopathology, and enhances the efficacy of conventional anti-TB drug [9]. In view of these promising findings we plan to test whether the existing approved anti-diabetic drug, Metformin added to existing anti-TB regimen, with its defined effects on host cell functions could be repurposed for effective and faster treatment of TB as compared to the current standard of care anti-TB regimens.

The Primary objective of this study– the METRIF study- is to study the antibacterial activity, in terms of time to sputum culture conversion of a metformin containing anti-tuberculosis treatment (ATT) regimen instituted during the initial 8-weeks of treatment in patients with newly diagnosed sputum smear positive pulmonary TB. Time to positivity or detection (TTD) of *M.tb* in culture in metformin containing regimen will be compared with the control group given only ATT as a secondary objective. Secondary objectives of the study include three other aspects (i) the autophagy enhancing effect and host immune responses when metformin is given along with ATT, (ii) post-dosing serum concentration of anti-TB drugs and metformin, their interactions and the effect of genomics - on these parameters (pharmacokinetics and pharmacogenomics) and (iii) the safety and tolerability of metformin by measuring the incidence of Treatment Emergent Adverse Events.

Methods and Analysis

Study Design and oversight: METRIF is a multi-site, randomized, open-labelled, parallel arm, controlled clinical trial comparing the time to sputum culture conversion among patients

with pulmonary TB receiving ATT with metformin (experimental arm) compared to those receiving ATT alone (control arm). The study is randomizing 316 participants to one of the two treatment arms in a 1:1 allocation. The study is sponsored by the India TB Research Consortium of the Indian Council of Medical Research and Open Source Pharma Foundation and implemented by the National Institute of Research in Tuberculosis (NIRT), together with other specialized institutes. The study has been approved by the Institutional ethics committee of NIRT (NIRT-IEC ID: 2017030, dated 14th December 2017) and NARI (NARI EC / 2018-10 dated 16th February 2018) and will begin enrollment tentatively by 15th June 2018. .

Study setting: METRIF study will be implemented at three sites in India - NIRT, Chennai and its satellite centers in Madurai and Vellore; All India Institute of Medical Sciences (AIIMS), New Delhi, and, National AIDS Research Institute (NARI), Pune. These sites will recruit study participants from academic institutions / hospitals as well as community clinics.

Study patients and eligibility: Adult patients, previously untreated and newly diagnosed as pulmonary TB with at least 2 sputum smear sample, collected on two different occasions, positive for acid-fast bacilli and susceptible to rifampicin detected by cartridge-based nucleic acid amplification test will be eligible for the study. Detailed inclusion and exclusion criteria are provided in Table 1. Patients who meet these criteria at presentation and attending the identified study sites will be approached to participate in the study.

Table 1: Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
age 18 years and above	Is pregnant or breast feeding
Is willing to undergo HIV testing	Has extra-pulmonary TB or drug-resistant TB
Has body weight between 30kg - 65kg	Has body weight < 30 kgs or > 65 kgs

Has never been treated with multidrug anti-TB therapy for more than a week.	Has prior history of exposure to anti-TB treatment for more than a week
Is willing to use effective contraceptive method during the study period	Has history of liver disease or current amino alanine transferase greater than 3 times the upper limit of normal or total bilirubin concentration greater than 2.5 times the ULN
Is willing to attend treatment center for supervised treatment and remain within the study area limit	Is serology positive for hepatitis B virus surface antigen or hepatitis C virus antibody
Is willing to sign the informed consent form and adhere to trial procedures and follow-up	Has concomitant psychiatric illness or seizures
Consents for Home visits by the study team	Has concomitant diabetes mellitus or random blood sugar > 200mg or fasting blood sugar >140 mg/dl
	Has serum creatinine >1.2 mg/dL or blood urea >43 mg/dL

Study regimen and Drug dosing: Eligible patients who have provided written informed consent will be randomly assigned to one of the study regimen in a ratio of 1:1. Study informed consent will be obtained by the Principal investigator at the site or his/her nominee who is capable of answering all the trial-related questions from the participants. Study participation will last 6 months: during the first 2 months participants will receive the randomly assigned regimen of either daily ATT with metformin or only ATT.

1) Test regimen – Metformin + Isoniazid + Rifampicin+ Pyrazinamide + Ethambutol daily [2MetHREZ$_7$] or

2) Control regimen – Isoniazid+ Rifampicin + Pyrazinamide + Ethambutol daily [2HRZE$_7$]

After 2-months, all study participants in both the arms will receive the standard 4-month continuation phase of HRE daily. Treatment will be supervised and directly observed at health facility or by DOT provider. Metformin will be dosed as 500 mg once daily for the 1st week and then 1000mg once daily for the remaining period of 7 weeks. Dosing of the other anti-TB drugs will be based on weight bands as shown in table 2.

All drugs (H, R, E, Z and Met) used in the trial will be centrally procured, checked for their stated content by validated methods using High Performance Liquid Chromatography at NIRT Clinical pharmacology department, prior to dispatching it to the enrolling sites for administration to patients.

Table 2: Weight based dosing of anti-tuberculosis drugs

Weight Band	Isoniazid	Rifampicin	Pyrazinamide	Ethambutol
25-39 kg	150 mg	300 mg	800 mg	550 mg
40-54 kg	225 mg	450 mg	1200 mg	825 mg
55-69 kg	300 mg	600 mg	1600 mg	1100 mg
>=70 kg	375 mg	750 mg	2000 mg	1375mg

Treatment allocation **:** Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities in chest x-ray and highest sputum smear grading at baseline (<2 or ≥2). Separate randomization lists for each combination of strata for each site will be prepared in advance by an independent statistician, using varying block sizes. Allocation codes will be generated at the central site and

at the time of patient's admission to the study, the central statistician through e-mail will inform the site the regimen based on appropriate stratification factors.

Recruitment process: All newly diagnosed sputum smear positive pulmonary TB patients attending the chest clinics at the study sites will be screened for study eligibility. Table 3 details the various study procedures. At their first visit, the study will be explained, including the potential risks and benefits associated with participation. Informed consent will be obtained before any protocol-specific screening procedures are carried out. Consenting participants will undergo sputum testing for smear, culture (both solid and liquid media) and rifampicin resistance testing by GeneXpert. Blood samples will be obtained for HIV antibodies (unless the patient is already known to be HIV positive), liver and renal function tests and blood sugar levels. Patients will be re-assessed for eligibility when returning with their investigation results. Those patients who do not have rifampicin resistance and are willing to take part in the study, sign an enrolment consent form (or a thumb print in the presence of a witness, if illiterate), and are admitted to the study and randomized to one of the study regimens. During the first two months of treatment, all patients will undergo weekly sputum testing for *M.tb* by smear, liquid and solid cultures and sparse pharmacokinetics of ATT drugs and metformin. Intense pharmacokinetic study will be done in a subset of patients. Randomized patients have an additional blood investigation for immunological and autophagy biomarkers (T-cell, monocyte & dendritic cell functions both ex-vivo and following stimulation with TB antigens including PPD and ESAT-6/CFP-10, estimation of CRP, TNF-α, and other cytokines) pre and post metformin containing ATT.

Patients ineligible or unwilling to participate in the study will be referred to the national programme for treatment as per the existing guidelines.

Table 3 : Study schedule of enrolment, interventions, and assessments.

		STUDY PERIOD														
		ENROLMENT	ALLOCATION	INTENSIVE PHASE OF ATT (TRIAL PERIOD)								CONTINUATION PHASE OF ATT (POST-TRIAL PERIOD)				CLOSE OUT
I	ENROLMENT	Baseline (-D7)	W 0	W 1	W 2	W 3	W 4	W 5	W 6	W 7	W 8	M 3	M 4	M 5	M 6	M 7
1	Informed consent	x														
2	Eligibility Screen (including lab tests)	x														
3	Sputum Gene Xpert	x														
4	HIV, Hepatitis B & C	x														
II.	ALLOCATION		x													
III	TRIAL REGIMENS															
	Test Regimen (Metformin containing ATT)		x	x	x	x	x	x	x	x	x					
	Control regimen (Only ATT)			x	x	x	x	x	x	x	x					
	Continuation Phase of ATT (For both groups)											x	x	x	x	
IV	ASSESSMENTS															
1	Clinical Evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2	Sociological Assessment	x	x				x				x	x	x	x	x	x
3	Sputum smear & culture (MGIT, LJ)	x		x	x	x	x	x	x	x	x	x	x	x	x	
4	Urine Pregnancy tests / Sugar / Alb	x					x				x	x	x	x	x	
5	HbA1c	x									x				x	
6	CBC, LFT, RFT, Lactic acid , peripheral smear & chest x-ray	x					x				x				x	
7	Random blood sugar	x			x		x		x		x				x	
8	Pharmacokinetics & genomics						x				x					
9	Immunological studies		x								x				x	
10	Storage Plasma/cell sample		x				x				x				x	
V	OUTCOMES															x

Treatment delivery, compliance and retention: ATT will be administered under direct observation for 6-days of the week and supplied for the seventh day. The importance of adherence to the treatment schedule will be reinforced at each visit. If the patient misses drug doses in the intensive phase (IP) or continuation phase (CP), it will be compensated at the end of the respective phases over next 15 days, so that the patient receives 60 doses of the assigned regimen in the IP and 120 doses in the CP. Retention of the participants in the study will be facilitated through through treatment supporters and enablers. All treatment is ambulatory and delivered by dedicated directly observed therapy (DOT) supporters. Treatment adherence will be assessed through reviews of treatment card throughout the IP and CP and by accounting for the empty pill covers returned if the drugs are supplied for any reason. Treatment adherence will be enhanced by – reimbursing the transport costs and loss of wages incurred by the participant during the study visits. They will also receive food supplement every month during the entire study period and meals during prolonged study visits.

Concomitant medication while in the trial: Concomitant antibiotic treatments of any kind are discouraged during the period of study drug administration. During the course of the trial, short course (< 2 weeks) antimicrobial therapy may be permitted for concurrent illnesses. Concomitant non-antibiotic treatments should be kept to a minimum during the study. Only those considered necessary for the subject's welfare and are unlikely to interfere with the study medication, may be given at the discretion of the investigator. The following agents used to treat *M. tb* infections should not be used during the trial: Streptomycin, Thiacetazone, PAS, Dapsone, Amoxicillin clavulinic acid/ clavulanate, Clofazimine, Capreomycin, any oxazolidinone antibiotic (e.g., linezolid), Ofloxacin, Levofloxacin or Moxifloxacin.

Criteria for Discontinuation/ Withdrawal of study participants: A patient may be withdrawn from the study for any of the following reasons – Pregnancy, if the investigators feel that staying in the study is harmful to the patient, if the patient does not follow study procedures or is not available for appointments, if the study sponsor or NIRT or representative of the Drugs Controller General of India decide to stop or cancel the study, if the Data and Safety Monitoring Board recommends that the study should be stopped or if the patient wishes to withdraw for any reasons. In such cases, appropriate treatment, as per the standard of care treatment available then will be ensured for patients taking into consideration the drug susceptibility pattern of the individual.

Study Outcomes: The primary outcome is the time to sputum culture conversion, which is assessed by the time interval between the date of treatment initiation and the date of acquisition of the first of at least two consecutive negative cultures taken at least 8 days apart. Also time to positivity as well as change in *M.tb* $\log_{10}$ colony forming units (CFU) in culture will be assessed in the two treatment arms of the study. Proportion of participants with sputum culture negativity will also be determined on a weekly basis between the two arms.

The pharmacokinetic (PK), area under the concentration curve (AUC), will be assessed through blood sampling on a single day during the first month of ATT, after a minimum of seven doses of RMP and MET (1000mg). Minimum inhibitory concentration (MIC) of each participant's pre-treatment infecting isolate will be estimated from early morning and overnight sputum samples collected at the pre-treatment visit. Genomics results of Metformin and Rifampicin will be compared with the plasma concentration of these drugs and will be associated with bacteriological and clinical endpoints along with drug-drug interaction of metformin and rifampicin.

For the occurrence of Treatment Emergent Adverse Events (TEAEs), clinical and laboratory abnormalities will be graded by clinical investigators according to the modified Adult Toxicity Table for the Division of Microbiology and Infectious Diseases, National Institutes of Health [11]. The safety and tolerability analysis will include all patients who were randomized to and received at least one dose of the study regimen.

Participant Timeline: The trial will consists of three stages - Screening and enrolment (a maximum of 1 week); Intensive phase of treatment with or without test drug (2 months) followed by continuation phase of standard drugs (4 months). Figure 1 shows the schedule of enrolment, interventions and assessments of participants in the trial.

Patient and public involvement: Patients were not involved in the development of research question or the design of this study as the scientific question is still not proven and only in research arena. However, considering the relevance of the study outcome to public health, policy-makers, general public, advocates etc are members of our scientific advisory committee and Institutional Ethics committee. Also the study will be discussed in our Community Advisory Board of the institute which consists of patients, peers and responsible members of the society. Patients will be involved in the recruitment and conduct of the study. Results of the study will be widely disseminated through meetings, workshops, conference presentation and publication

Sample Size Assumption: Published literature has shown a median time to sputum culture conversion by liquid medium with daily ATT to be 32 days [10]. With addition of metformin, we assumed a 30% reduction in the time to culture conversion i.e., to approximately 22 days. We estimated that we will require 150 new sputum smear positive patients to show this difference at 80% power, an alpha level of 0.05 and a hazards ratio of 1.5. With the assumption that 5% of

patients will be lost to follow-up or not assessable in the primary analysis, a total target sample size of 158 patients in each treatment arm would be required, totaling to 316 patients for the study.

Randomization Procedures: Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities on chest x-ray and highest sputum smear grading (<2 or >2). The randomization sequence will not be available to those who enroll participants. Allocation codes will be generated at the central site, NIRT and will take place at the time of patient's admission to the study. Upon receiving an electronic-mail request from the study sites, for allocation of a participant to the study, the NIRT statistician will do allocation procedure centrally based on appropriate stratification factors. He/She will then inform the site physician, through e-mail, the study regimen along with the unique study ID for patient enrolment to the study.

Data Collection, Management and Interim Analysis: Study randomization will be done centrally by the Central Data management (CDM) unit at NIRT, Chennai over e-mail. During the study conduct, data will be collected at sites at baseline and follow-up on pre-defined case record forms and will be electronically transmitted to the CDM unit. Data will be checked for correctness and completeness before transmitting. Periodic quality check of the data will also be done by the central team. Data collected will include a) improvement in disease status in terms of abetment of symptoms, signs and sputum cultures conversion and b) safety and tolerability of the regimen in terms of both clinical and laboratory adverse events.

During the trial all essential trial documents including the source documents, informed consent forms, etc. will be stored securely under lock and key at the recruiting sites under the supervision of the site investigator. All e-data will be password protected with limited access to

the investigator and their teams alone. The records will be retained for a minimum period of five years after completion of the study.

Two interim analyses are planned during the study period, viz. after 33% and 66% of the enrolled patients have completed 8-weeks of Metformin treatment and with sputum culture results available for analysis. In addition, interim analysis may be done if the frequency of reported serious adverse events is greater than anticipated. Final analysis will be done when all enrolled patients have completed 6 months of ATT.

Study Outcome analysis: The primary efficacy analysis will compare the median time to culture negativity. This analysis will be done when the last enrolled patient has completed 8 weeks of treatment and will be done using culture results from liquid culture (MGIT) and in those who are not isoniazid or RMP mono-resistant at baseline. Both a Modified Intent to Treat (MITT) and a Per Protocol (PP) analysis will be conducted. Secondary outcome parameters will include proportion and time to sputum culture positivity using the MGIT system. The safety and tolerability analysis will include all patients who were randomized to and received at least one dose of the drug. Based on plasma drug concentrations obtained at different time points, certain pharmacokinetic variables will be calculated. Drug peak concentrations (Cmax) and exposure (AUC) will be related to time to sputum conversion and occurrence of adverse events

Data Safety Monitoring Board (DSMB): The DSMB, consisting of TB clinicians, pharmacokinetic specialists, and an independent biostatistician, will review data of this trial on a regular basis. The role of the DSMB will be to review the progress of the trial, and detect evidence of early safety issues from the data collected, for the trial participants, while the trial is ongoing. The following safety parameters will be analyzed for the regular DSMB meetings: incidence and severity of all AEs with specific focus on grade 3 or 4 AEs, SAEs, and treatment

discontinuations due to AE. Based on the results, DSMB shall make recommendations on continuing or terminating / modifications to the trial.

Clinical Site Monitoring & Quality assurance: An independent study Monitor, appointed by the sponsor, will be responsible for monitoring data quality in accordance with trial Standard Operating Procedures. Based on the monitoring plan, field visit and audit will be performed at different stages. All participant records, CRFs, and other source documents for the patients recruited in this study will be made available for review by the monitors. A meeting of the investigators of each local site will convene monthly via web-based remote conference system to share the progress of study and discuss with the problems met during the trial conduct. During the conduct of the study, any important protocol modifications will be informed to the IEC, trial registry and if relevant to the trial participants.

Confidentially of trial data: The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the anti-bacterial activity, safety, and tolerability of the investigational product used in this trial. These data will be processed with adequate precautions to ensure confidentiality. The data will be collected by trained professionals with utmost sensitivity and confidentiality. The study participant will be informed during the informed consent process that - the monitor(s), the IEC, and the regulatory authority(ies) will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the participant, to the extent permitted by the applicable laws. Otherwise, only the study investigator and his/her team will have access to the trial data.

Ethics and Dissemination

This trial will be conducted in accordance with the current ICH Good Clinical Practice and the ICMR Ethical guidelines for biomedical research in human participants. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected; consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible. The confidentiality of the study participants will be protected throughout the study period as per provision in the Indian-GCP & applicable regulations by laws of India. The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the anti-bacterial activity, safety, and tolerability of the investigational product used in this trial.

The study principal investigator holds primary responsibility for the preparation of manuscripts and materials for result dissemination and publication. Once the trial is complete, the investigators anticipate publishing results of this study in several peer-reviewed scientific journals, presenting the abstracts in meetings, to stakeholders and policy makers and share the results widely with the Programme managers. Trial participant details will not be shared during any of these processes.

Discussions:

Currently, for the management of new sputum smear positive drug-sensitive pulmonary TB patients, 6-month regimens are used in more than 90 countries that are evaluated to be effective [12]. Newer anti-TB drugs are also in pipeline. However, all types of treatment currently available are pathogen-targeted. They are also associated with drug-toxicity which leads to poor adherence to treatment resulting in treatment failure and development of resistance.

As the pathogen-targeted strategies may lead to development of acquired microbial resistance, new "host-targeted" adjunct therapeutic strategies not only are less likely to engender microbial resistance but also augment protective host immune responses thus accelerating bacterial clearance from the system.

Metformin has an inhibitory effect on mitochondrial complex I, inhibition of which has been found to increase the AMP/ATP ratio [13, 14]. The altered cellular energy status induces activation of AMP-activated protein kinase (AMPK), a serine/threonine kinase, and acts as an energy sensor [15]. Activation of AMPK by metformin stimulates endothelial nitric oxide synthase (eNOS) activity which leads to bacterial killing [16]. Metformin also acts through AMPK-independent mechanisms. It promotes phagocytosis, phago-lysosome fusion & autophagy in macrophages. Macrophages exposed to metformin had higher bactericidal capacity attributed to increased mitochondrial Reactive Oxidative Species (ROS) production required for bacterial killing [17]. Since 90% of the newly diagnosed sputum positive patients are sensitive to isoniazid (H) and rifampicin (R), it is assumed that adding the drug metformin would have an added beneficial effect in terms of early killing of intracellular bacteria by influencing the host immunity. Experiments in mice with MET (500 mg/kg) alone have shown reduced bacillary load in both lung and spleen which is equivalent to a MET dose of 2430 mg/day for a 60-kg human [18]. High dose of metformin has also been used in clinical practice for management of diabetic individuals with and without TB and also in non-diabetic conditions like polycystic ovarian syndrome and obesity [19, 20].

We are proposing a Phase IIB trial looking at sputum-culture conversion to negative over a 2-month period as studies have shown a correlation between positive 2-month sputum-culture status and subsequent relapse. However, limitations of this design include the binary outcome of

2-month sputum-culture end point, hence requiring larger samples size to prove the benefit. To overcome this limitation we plan serial sputum colony counting (SSCC) and time-to-detection (TTD). SSCC employs quantitative sputum cultures measured at several time points over a 2-month period. These measurements allow for the calculation of time to stable culture conversion as well as change in CFU/ml/day, a longitudinal continuous variable with greater power compared with the binary culture conversion [21]. TTD in liquid culture may offer a potential alternative to SSCC and the problems of quantitative cultures, by replacing CFU counting with the automated measurement of TTD during the 8-week period. In our study, the outcome measures of interest (primarily bacteriological) is based on objective microbiology and the bacteriology laboratory staff will be blinded to the patient's treatment. Safety assessments will be defined as objectively as possible, using pre-defined grading criteria for laboratory abnormalities and adverse events. The pharmacokinetic aspect of the study is based on a population approach which is facilitated by the intensive-sparse sampling design thus enabling pharmacokinetic exposure (AUC0–24) to be estimated for all participants in the study. Several alternative biomarkers of treatment response are also being evaluated using these samples.

Results from METRIF study will complement observations from other retrospective and case-control studies that showed metformin to be a protective agent against TB infection among diabetics [22, 23]. As studies did not show any dose-dependent protection in metformin users for TB, we will be using 500mg dose for the first week followed by 1000mg for the remaining 7-weeks. This dose escalation is being done to reduce the gastrointestinal side effects of metformin. If the study regimen is successful, it will pave way to evaluate shorter regiments for the treatment of PTB. This is expected to provide a new standard of care for DS-PTB which would cut down the number of required clinic visits and the burden on the health care system and

could also decrease the percentage of patients who fail to complete the full course of current longer therapy. If the study regimens are shown to be non-inferior or superior to the control regimen, that would represent an even greater advance for patients with DS-PTB and TB control programmes globally.

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- ***Competing interests***: None

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	15
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6 & 7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12& 20

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	-
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___13_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	____-_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____13_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____12_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_13 & 14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____-_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____6_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____14_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Yes
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Yes

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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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

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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

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Abstract

Introduction: Shorter duration of treatment for the management of drug-susceptible pulmonary tuberculosis (TB) would be a significant improvement in the care of patients suffering from the disease. Besides newer drugs and regimens, other modalities like host-directed therapy are also being suggested to reach this goal. This study's objective is to assess the efficacy and safety of metformin-containing anti-tuberculosis treatment (ATT) regimen in comparison to the standard 6-month ATT regimen in the treatment of newly diagnosed sputum smear positive drug-sensitive pulmonary tuberculosis patients.

Methods and Analysis: We are conducting a multicentric, randomized open-label controlled clinical trial to achieve the study objective. The intervention group will receive isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) along with 1000mg of daily metformin (Met) for the first two months while the control group will receive only HRZE. After 2-months, both the groups will receive HRE daily for four months. The primary endpoint is time to sputum culture conversion. Secondary endpoints will include time to detection of *mycobacterium tuberculosis* in sputum, pharmacokinetics, and pharmacogenomics of study drugs, drug-drug interactions, safety and tolerability of the various combinations and measurement of autophagy and immune responses in the study participants.

Ethics and Dissemination: The Ethics committee of the participating institutes have approved the study. Results from this trial will contribute to evidence towards constructing a shorter, effective and safe regimen for TB patients. The results will be shared widely with the National Programme managers, policymakers, and stakeholders through open access publications, dissemination meetings, conference abstracts and policy briefs. This is expected to provide a new

standard of care for drug-sensitive pulmonary TB patients who will not only reduce the number of clinic visits and lost to follow-up of patients from treatment but also reduce the burden on the health care system.

Trial Registration: The protocol has been registered on Clinical Trial Registry of India with identifier CTRI/2018/01/011176 on January 8th, 2018.

Strength and Limitations:

- Study design, randomized controlled clinical trial, will add considerable power to the study results and conclusion by decreasing bias (selection bias & observer bias) and minimizing confounding of unequal distribution.
- This study design of randomized controlled clinical trial with stratification will also control of group allocation, enhancing similarity of baseline features including disease severity in the two study arms. This will help us in subgroup analysis improving usefulness for clinical practice
- Due to the strict exclusion and inclusion criteria of the clinical trial, the results from the clinical trial may not be generalizable to all groups of TB patients.
- Due to the multicentric nature of the study, the heterogeneity of data may be present
- Relapse rates may not be known in this trial as the study duration is only for six months of treatment as per the study objective

Keywords: Metformin, Pulmonary Tuberculosis, Clinical trial, Short-course therapy, TB treatment

Introduction:

Globally in 2016, there were an estimated 10.4 million new cases of tuberculosis (TB) with five countries, India, Indonesia, China, Philippines, and Pakistan accounting for 56% of the total cases [1]. There were an estimated 1.3 million TB deaths in 2016 among HIV-negative people and an additional 374000 deaths among people living with HIV [1]. Though effective regimens are available for the treatment of drug-sensitive TB with more than 95% cure, the long duration of such regimens has posed problems for TB treatment and control. This, along with drug toxicity results in poor adherence to treatment resulting in the emergence of drug resistance.

The global need for new effective therapies has led to a resurgence in efforts to identify additional anti-*M.tb* agents, several of which are now being evaluated in clinical trials. However, conventional pathogen-targeted strategies suffer from the severe disadvantage of fostering microbial resistance. To circumvent this problem, a new paradigm in drug discovery has emerged that involves therapeutic modulation of the host immune responses which leads to better and faster pathogen elimination [2, 3].

A competent host immune system is a crucial factor for both the control and containment of *M.tb* growth. *M.tb* can evade host innate as well as adaptive immune responses and succeed in infecting the host cells and maintaining long-term persistent infection [4, 5]. “Host-targeted” adjunct therapeutic strategies not only augment protective host immune responses but also reduce the chance of development of microbial resistance.

One among the host cell innate antimicrobial arsenal includes the capacity to destroy intracellular pathogens using the phagosomal machinery or autophagy pathway. Intracellular pathogens are effectively controlled by autophagy that is regulated by adenosine monophosphate-activated protein kinase (AMPK) [6, 7]. The *M.tb* virulence results from

perturbations in the autophagy network and AMPK signaling [8]. The antidiabetic drug Metformin (MET; 1,1-dimethyl biguanide), is an AMPK modulator that inhibits the intracellular growth of *M.tb*, restricts disease immunopathology, and enhances the efficacy of conventional anti-TB drug [9]. Given these promising findings, we plan to test whether the existing approved anti-diabetic drug, Metformin added to existing anti-TB regimen, with its defined effects on host cell functions could be repurposed for effective and faster treatment of TB as compared to the current standard of care anti-TB regimens.

The Primary objective of this study– the METRIF study- is to study the antibacterial activity, in terms of time to sputum culture conversion of metformin-containing anti-tuberculosis treatment (ATT) regimen instituted during the initial 8-weeks of treatment in patients with newly diagnosed sputum smear-positive pulmonary TB. Secondary objectives of the study are:

i. to compare the time to detection (TTD) of *M.tb* in culture in the group receiving metformin-containing regimen with the control group receiving ATT alone.

ii. To study the autophagy enhancing effect and host immune responses in the two groups,

iii. To examine the post-dosing serum concentration of anti-TB drugs and metformin, their interactions and the impact of genomics - on these parameters (pharmacokinetics (PK) and pharmacogenomics) and

iv. To evaluate the safety and tolerability of metformin by measuring the incidence of Treatment-Emergent Adverse Events.

Methods and Analysis

Study Design and oversight: METRIF is a multi-site, randomized, open-labeled, parallel arm, controlled clinical trial comparing the time to sputum culture conversion among patients with pulmonary TB receiving ATT with metformin (experimental arm) compared to those receiving ATT alone (control arm). The study is randomizing 316 participants to one of the two treatment arms in a 1:1 allocation. The study is sponsored by the India TB Research Consortium of the Indian Council of Medical Research and Open Source Pharma Foundation and implemented by the National Institute of Research in Tuberculosis (NIRT), together with other specialized institutes. The Institutional ethics committee of NIRT has approved the study (NIRT-IEC ID: 2017030, dated 14th December 2017) and National AIDS Research Institute (NARI) (NARI EC / 2018-10 dated 16th February 2018) and will begin enrollment tentatively by 15th June 2018.

Study setting: We will implement METRIF study at three sites in India - NIRT, Chennai and its satellite centers in Madurai and Vellore; All India Institute of Medical Sciences, New Delhi, and, NARI, Pune. These sites will recruit study participants from academic institutions/hospitals as well as community clinics.

Study patients and eligibility: Adult patients, previously untreated and newly diagnosed as pulmonary TB with at least two sputum smear sample, collected on two different occasions, positive for acid-fast bacilli and susceptible to rifampicin detected by cartridge-based nucleic acid amplification test will be eligible for the study. Table 1 provides the detailed inclusion and exclusion criteria. Patients who meet these criteria at presentation and attending the identified study sites will be approached to participate in the study.

Table 1: Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
age 18 years and above	Is pregnant or breastfeeding
Is willing to undergo HIV testing	Has extra-pulmonary TB or drug-resistant TB
Has body weight between 30kg - 65kg	Has body weight < 30 kgs or > 65 kgs
Patient has never received treatment with multidrug anti-TB therapy for more than a week.	Has a prior history of exposure to anti-TB treatment for more than a week
Is willing to use an effective contraceptive method during the study period	Has a history of liver disease or current amino alanine transferase greater than three times the upper limit of normal or total bilirubin concentration greater than 2.5 times the ULN
Is willing to attend a treatment center for supervised treatment and remain within the study area limit	Is serology positive for hepatitis B virus surface antigen or hepatitis C virus antibody
Is willing to sign the informed consent form and adhere to trial procedures and follow-up	Has concomitant psychiatric illness or seizures
Consents for Home visits by the study team	Has concomitant diabetes mellitus or random blood sugar > 200mg or fasting blood sugar >140 mg/dl
	Has serum creatinine >1.2 mg/dl or blood urea >43 mg/dl

Study regimen and Drug dosing: We will randomly assign eligible patients who have provided written informed consent to one of the study regimens in a ratio of 1:1. The site Principal Investigator or his/her nominee who is capable of answering all the trial-related questions from the participants will obtain the study consent. Study participation will last six months: during the first two months, participants will receive the randomly assigned regimen of either daily ATT with metformin or only ATT.

1) Test regimen – Metformin + Isoniazid + Rifampicin+ Pyrazinamide + Ethambutol daily [$2MetHREZ_7$] or

2) Control regimen – Isoniazid+ Rifampicin + Pyrazinamide + Ethambutol daily [$2HRZE_7$]

After 2-months, all study participants in both the arms will receive the standard 4-month continuation phase of HRE daily. Treatment will be supervised and directly observed at a health facility or by directly observed therapy (DOT) provider. Metformin will be dosed at 500 mg once daily for the 1^{st} week and then 1000mg once daily for the remaining period of 7 weeks. Dosing of the other anti-TB drugs will be based on weight bands as shown in table 2.

We will centrally procure all drugs (H, R, E, Z, and Met) to be used in the trial, check for their stated content by validated methods using High-Performance Liquid Chromatography at NIRT Clinical pharmacology department, before dispatching it to the enrolling sites for administration to patients.

Table 2: Weight-based dosing of anti-tuberculosis drugs

Weight Band	Isoniazid	Rifampicin	Pyrazinamide	Ethambutol
30-39 kg	150 mg	300 mg	800 mg	550 mg
40-54 kg	225 mg	450 mg	1200 mg	825 mg
55-65 kg	300 mg	600 mg	1600 mg	1100 mg

Treatment allocation: Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities in chest x-ray and highest sputum smear grading at baseline (<2 or ≥2). Separate randomization lists for each combination of strata for each site will be prepared in advance by an independent statistician, using varying block sizes. Allocation codes will be generated at the central location and at the time of patient's admission to the study, the primary statistician through e-mail will inform the site the regimen based on appropriate stratification factors.

Recruitment process: We will screen all newly diagnosed sputum smear-positive pulmonary TB patients attending the chest clinics at the study sites for study eligibility. Table 3 details the various study procedures. At their first visit, the study will be explained, including the potential risks and benefits associated with participation. We will obtain informed written consent before any protocol-specific screening procedures are carried out. Consenting participants will undergo sputum testing for smear, culture (both solid and liquid media) and rifampicin resistance testing by GeneXpert. Blood samples will be obtained for HIV antibodies (unless the patient is already known to be HIV positive), liver and renal function tests and blood sugar levels. Patients will be re-assessed for eligibility when returning with their investigation results. Those patients who do not have rifampicin resistance and are willing to take part in the study will sign an enrolment consent form (or a thumb-print in the presence of a witness, if illiterate), and randomized to one of the study regimens. During the first two months of treatment, all patients will undergo weekly sputum testing for *M.tb* by smear, liquid, and solid cultures and sparse pharmacokinetics of ATT drugs and metformin. A subset of patients will undergo intense pharmacokinetic study. Randomized patients have an additional blood investigation for immunological and autophagy biomarkers (T-cell, monocyte & dendritic cell

functions both ex-vivo and following stimulation with TB antigens including PPD and ESAT-6/CFP-10, estimation of CRP, TNF-α, and other cytokines) pre and post metformin containing ATT.

We will refer patients ineligible or unwilling to participate in the study to the national programme for treatment as per the existing guidelines.

Table 3: Study schedule of enrolment, interventions, and assessments.

		STUDY PERIOD														
		ENROLMENT	ALLOCATION	INTENSIVE PHASE OF ATT (TRIAL PERIOD)								CONTINUATION PHASE OF ATT (POST-TRIAL PERIOD)				CLOSE OUT
I	ENROLMENT	Baseline (-D7)	W 0	W 1	W 2	W 3	W 4	W 5	W 6	W 7	W 8	M 3	M 4	M 5	M 6	M 7
1	Informed consent	x														
2	Eligibility Screen (including lab tests)	x														
3	Sputum Gene Xpert	x														
4	HIV, Hepatitis B & C	x														
II.	ALLOCATION		x													
III	TRIAL REGIMENS															
	Test Regimen (Metformin containing ATT)		x	x	x	x	x	x	x	x	x					
	Control regimen (Only ATT)			x	x	x	x	x	x	x	x					
	Continuation Phase of ATT (For both groups)											x	x	x	x	
IV	ASSESSMENTS															
1	Clinical Evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2	Sociological Assessment	x	x				x				x	x	x	x	x	x
3	Sputum smear & culture (MGIT, LJ)	x		x	x	x	x	x	x	x	x	x	x	x	x	
4	Urine Pregnancy tests / Sugar / Alb	x					x				x	x	x	x	x	
5	HbA1c	x									x				x	
6	CBC, LFT, RFT, Lactic acid , peripheral smear & chest x-ray	x					x				x				x	
7	Random blood sugar	x			x		x		x		x				x	
8	Pharmacokinetics & genomics						x				x					
9	Immunological studies		x								x				x	
10	Storage Plasma/cell sample		x				x				x				x	
V	OUTCOMES															x

Treatment delivery, compliance, and retention: ATT will be administered under direct observation for 6-days of the week and supplied for the seventh day. Study staff will reinforce the importance of adherence to the treatment schedule at each visit. If the patient misses drug doses in the intensive phase (IP) or continuation phase (CP), it will be compensated at the end of the respective stages over next 15 days, so that the patient receives 60 doses of the assigned regimen in the IP and 120 doses in the CP. Treatment supporters and enablers will facilitate retention of the participants in the study. All treatment is ambulatory and delivered by dedicated DOT provider. We will assess treatment adherence through reviews of treatment card throughout the treatment phase. In case of drug supply for any reason, empty pill cover count will determine adherence. Treatment adherence will be enhanced by – reimbursing the transport costs and loss of wages incurred by the participant during the study visits. During the study period, participants will receive food supplement every month and meals during their extended, study visits.

Concomitant medication while in the trial: Concomitant antibiotic treatments of any kind are discouraged during the study period. If required, their usage is restricted for a short course (< 2 weeks) period. Concomitant non-antibiotic drug usage is allowed in small quantity. Only those considered necessary for the subject's welfare and are unlikely to interfere with the study medication, may be given at the discretion of the investigator. Below are the drugs used to treat *M. tb* infections which should not be used during this trial: Streptomycin, Thiacetazone, PAS, Dapsone, Amoxicillin-clavulanic acid/ clavulanate, Clofazimine, Capreomycin, any oxazolidinone antibiotic (e.g., linezolid), Ofloxacin, Levofloxacin or Moxifloxacin.

Criteria for Discontinuation/ Withdrawal of study participants: Withdrawal of study participants can occur for any of the following reasons – Pregnancy, if the investigators feel that staying in the study is harmful to the patient, if the patient does not follow study procedures or is

not available for appointments, if the study sponsor or NIRT or representative of the Drugs Controller General of India decide to stop or cancel the study, if the Data and Safety Monitoring Board recommends halting the study or if the patient wishes to withdraw for any reasons. In such cases, appropriate treatment, as per the standard of care treatment available then will be ensured for patients taking into consideration the drug susceptibility pattern of the individual.

Study Outcomes: The primary outcome is the time to sputum culture conversion, which is assessed by the time interval between the date of treatment initiation and the date of acquisition of the first of at least two consecutive negative cultures taken at least eight days apart. We will also assess, on a weekly basis, the time to positivity, a change in *M.tb* $\log_{10}$ colony forming units (CFU) in culture and the proportion of participants with sputum culture negativity in the two treatment arms.

The PK, the area under the concentration curve (AUC), will be assessed through blood sampling on a single day during the first month of ATT, after a minimum of seven doses of RMP and MET (1000mg). We will estimate the Minimum inhibitory concentration (MIC) of each participant's pre-treatment infecting isolate from early morning and overnight sputum samples collected at the pre-treatment visit. We will also compare the Genomics results of Metformin and Rifampicin with the plasma concentration of these drugs, bacteriological and clinical endpoints along with the drug-drug interaction of metformin and rifampicin.

For the occurrence of Treatment-Emergent Adverse Events (TEAEs), clinical investigators will grade clinical and laboratory abnormalities according to the modified Adult Toxicity Table for the Division of Microbiology and Infectious Diseases, National Institutes of Health [10]. The safety and tolerability analysis will include all patients who were randomized to and received at least one dose of the study regimen.

Participant Timeline: The trial will consist of three stages - Screening and enrolment (a maximum of 1 week); the Intensive phase of treatment with or without test drug (2 months) followed by a continuation phase of standard drugs (4 months). Table 3 shows the schedule of enrolment, interventions, and assessments of participants in the trial.

Patient and public involvement: Patients were not involved in the development of the research question, or the design of this study as the scientific problem is still not proven and only in the research arena. However, considering the relevance of the study outcome to public health, and policy-makers, the study is discussed in our Institutional Scientific advisory committee, Institutional Ethics committee and Community Advisory Board consisting of a representative from affected community, peers and responsible members of the society. Patients will be involved in the recruitment and conduct of the study. We will disseminate the results of the study widely through meetings, workshops, conference presentation, and publication

Sample Size Assumption: Published literature has shown a median time to sputum culture conversion by a liquid medium with daily ATT to be 32 days [11]. With the addition of metformin, we assumed a 30% reduction in the time to culture conversion, i.e., to approximately 22 days. We estimated that we would require 150 new sputum smear-positive patients to show this difference at 80% power, an alpha level of 0.05 and a hazards ratio of 1.5. With the assumption that 5% of patients will be lost to follow-up or not assessable in the primary analysis, a total target sample size of 158 patients in each treatment arm will be recruited, totaling to 316 patients for the study.

Randomization Procedures: Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities on chest x-ray and highest sputum smear grading (<2 or >2). The randomization sequence will not

be available to those who enroll participants. Allocation codes will be generated at the central site, NIRT and will take place at the time of patient's admission to the study. Upon receiving an electronic-mail request from the study sites, for allocation of a participant to the study, the NIRT statistician will do allocation procedure centrally based on appropriate stratification factors. He/She will then inform the site physician, through e-mail, the study regimen along with the unique study ID for patient enrolment to the study.

Data Collection, Management, and Interim Analysis: Study randomization will be done centrally by the Central Data management (CDM) unit at NIRT, Chennai over e-mail. During the study conduct, data will be collected at sites at baseline and follow-up on pre-defined case record forms (CRF) and transmitted electronically to the CDM unit. Data correctness and completeness will be checked before sending. The central team will also conduct a periodic quality check of the data. Data collected will include a) improvement in disease status regarding abetment of symptoms, signs and sputum cultures conversion and b) safety and tolerability of the regimen concerning both clinical and laboratory adverse events.

During the trial all essential trial documents including the source documents, informed consent forms, etc. will be stored securely under lock and key at the recruiting sites under the supervision of the site investigator. All e-data will be password protected with limited access to the investigator and their teams alone. The study team will retain the records for a minimum period of five years after completion of the study.

The study has two interim analyses planned, viz. after 33% and 66% of the enrolled patients have completed 8-weeks of Metformin treatment and with sputum culture results available for review. The study also has an additional interim analysis for reported serious

adverse events with frequency higher than anticipated. The final analysis will include all enrolled patients when they have completed six months of ATT.

Study Outcome analysis: The primary efficacy analysis will compare the median time to culture negativity. This analysis will happen when the last enrolled patient has completed eight weeks of treatment and will be done using culture results from liquid culture (MGIT) and in those who are not isoniazid or RMP mono-resistant at baseline. Both a Modified Intent to Treat (MITT) and a Per Protocol (PP) analysis will be conducted. Secondary outcome parameters will include proportion and time to sputum culture positivity using the MGIT system. The safety and tolerability analysis will consist of all patients who were randomized to and received at least one dose of the drug. Based on plasma drug concentrations obtained at different time points, we will calculate certain pharmacokinetic variables related to study drugs. Drug peak concentrations (Cmax) and exposure (AUC) will be linked to time to sputum conversion and occurrence of adverse events

Data Safety Monitoring Board (DSMB): The DSMB for this study comprises of TB clinicians, pharmacokinetic specialists, and an independent biostatistician. They will review data from this trial on a regular basis, including incidence progress of the trial, and detect evidence of early safety issues for the trial participants with a specific focus on grade 3 or 4 AEs, SAEs, and treatment discontinuations due to AE. Based on the results, DSMB shall make recommendations on continuing or terminating / modifications to the trial.

Clinical Site Monitoring & Quality assurance: An independent study Monitor, appointed by the sponsor, will be responsible for monitoring data quality by trial Standard Operating Procedures. Based on the monitoring plan, field visit and audit will be performed at different stages. All participant records, CRFs, and other source documents for the patients recruited in

this study will be made available for review by the monitors. A meeting of the investigators of each local site will convene monthly via web-based remote conference system to share the progress of the study and discuss with the problems met during the trial conduct. During the conduct of the research, any critical protocol modifications will be informed to the IEC, trial registry and if relevant to the trial participants.

Confidentially of trial data: The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the antibacterial activity, safety, and tolerability of the investigational product used in this trial. These data will be processed with adequate precautions to ensure confidentiality. Trained professional will collect al study data with the utmost sensitivity and confidentiality. The study participant will be informed during the informed consent process that - the monitor(s), the IEC, and the regulatory authority(is) will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the participant, to the extent permitted by the applicable laws. Otherwise, only the study investigator and his/her team will have access to the trial data.

Ethics and Dissemination

This trial will proceed as per the current ICH Good Clinical Practice and the ICMR Ethical guidelines for biomedical research in human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial subjects are protected; consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible. The confidentiality of the study participants will be protected throughout the study period as per provision in the Indian-GCP & applicable regulations by laws

of India. The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the antibacterial activity, safety, and tolerability of the investigational product used in this trial.

The study principal investigator holds primary responsibility for the preparation of manuscripts and materials for result dissemination and publication. Once the trial is complete, the investigators anticipate publishing results of this study in several peer-reviewed scientific journals, present the abstracts in meetings, to stakeholders and policymakers and share the results widely with the Programme managers. During all these processes, details of the trial participant will remain confidential.

Discussions:

Currently, for the management of new sputum smear-positive drug-sensitive pulmonary TB patients, 6-month regimens are used in more than 90 countries that are evaluated to be effective [12]. Newer anti-TB drugs are also in the pipeline. However, all types of treatment currently available are pathogen-targeted and with toxic drugs. Drug-toxicity can lead to poor treatment adherence resulting in treatment failure and development of resistance. As the pathogen-targeted strategies may lead to the development of acquired microbial resistance, new "host-targeted" adjunct therapeutic approaches not only are less likely to engender microbial resistance but also augment protective host immune responses thus accelerating bacterial clearance from the system.

Metformin has an inhibitory effect on mitochondrial complex I, inhibition of which has been found to increase the AMP/ATP ratio [13, 14]. The altered cellular energy status induces activation of AMPK, a serine/threonine kinase, and acts as an energy sensor [15]. Activation of

AMPK by metformin stimulates endothelial nitric oxide synthase (eNOS) activity which leads to bacterial killing [16]. Metformin also acts through AMPK-independent mechanisms. It promotes phagocytosis, phagolysosome fusion & autophagy in macrophages. Macrophages exposed to metformin had higher bactericidal capacity attributed to increased mitochondrial Reactive Oxidative Species (ROS) production required for bacterial killing [17]. Since 90% of the newly diagnosed sputum positive patients are sensitive to isoniazid (H) and rifampicin (R), it is assumed that adding the drug metformin would have a beneficial effect in the early killing of intracellular bacteria by influencing the host immunity. Experiments in mice with MET (500 mg/kg) alone have shown reduced bacillary load in both lung and spleen which is equivalent to a MET dose of 2430 mg/day for a 60-kg human [18]. High dose of metformin has also been used in clinical practice for the management of diabetic individuals with and without TB and in non-diabetic conditions like polycystic ovarian syndrome and obesity [19, 20].

We are proposing a Phase IIB trial looking at sputum-culture conversion to negative over a 2-month period as studies have shown a correlation between positive 2-month sputum-culture status and subsequent relapse. However, limitations of this design include the binary outcome of 2-month sputum-culture endpoint, hence requiring larger samples size to prove the benefit. To overcome this limitation, we plan serial sputum colony counting (SSCC) and time-to-detection (TTD). SSCC employs quantitative sputum cultures measured at several time points over a 2-month period. These measurements allow for the calculation of time to stable culture conversion as well as a change in CFU/ml/day, a continuous longitudinal variable with greater power compared with the binary culture conversion [21]. TTD in liquid culture may offer a potential alternative to SSCC and the problems of quantitative cultures, by replacing CFU counting with the automated measurement of TTD during the 8-week period. In our study, as the outcome

measures of interest (primarily bacteriological) are based on objective microbiology, the bacteriology laboratory staff will be blinded to the patient's treatment. Safety assessments will be defined as objectively as possible, using pre-defined grading criteria for laboratory abnormalities and adverse events. The pharmacokinetic aspect of the study is based on a population approach which is facilitated by the intensive-sparse sampling design. The study will estimate the pharmacokinetic exposure (AUC0–24) for all study participants. Several alternative biomarkers of treatment response are also being evaluated using these samples.

Results from METRIF study will complement observations from other retrospective and case-control studies that showed metformin to be a protective agent against TB infection among people with diabetes [22, 23]. As studies did not show any dose-dependent protection in metformin users for TB, we will be using 500mg dose for the first week followed by 1000mg for the remaining 7-weeks. This dose escalation is being done to reduce the gastrointestinal side effects of metformin. If the study regimen is successful, it will pave the way to evaluate shorter regiments for the treatment of PTB. This is expected to provide a new standard of care for DS-PTB which would cut down the number of required clinic visits and the burden on the health care system and could also decrease the percentage of patients who fail to complete the full course of current longer therapy. If the study regimens are shown to be non-inferior or superior to the control regimen, that would represent an even greater advantage for patients with DS-PTB and TB control programmes globally.

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- ***Authors' contributions***: CPP, SS, RG & TB – conceived & designed the study / CPP, PKB, MN, CPR, HK, NSG, MSJ, MS, & SS – development and writing of the study protocol and this manuscript

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- ***Competing interests***: None

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	15
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6 & 7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12& 20

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___13_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	____n/a_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____13_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____12_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_13 & 14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____n/a_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____6_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____14_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Yes
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Yes

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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

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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

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Abstract

Introduction: Shorter duration of treatment for the management of drug-susceptible pulmonary tuberculosis (TB) would be a significant improvement in the care of patients suffering from the disease. Besides newer drugs and regimens, other modalities like host-directed therapy are also being suggested to reach this goal. This study's objective is to assess the efficacy and safety of metformin-containing anti-tuberculosis treatment (ATT) regimen in comparison to the standard 6-month ATT regimen in the treatment of newly diagnosed sputum smear positive drug-sensitive pulmonary tuberculosis patients.

Methods and Analysis: We are conducting a multicentric, randomized open-label controlled clinical trial to achieve the study objective. The intervention group will receive isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) along with 1000mg of daily metformin (Met) for the first two months while the control group will receive only HRZE. After 2-months, both the groups will receive HRE daily for four months. The primary endpoint is time to sputum culture conversion. Secondary endpoints will include time to detection of *mycobacterium tuberculosis* in sputum, pharmacokinetics, and pharmacogenomics of study drugs, drug-drug interactions, safety and tolerability of the various combinations and measurement of autophagy and immune responses in the study participants.

Ethics and Dissemination: The Ethics committee of the participating institutes have approved the study. Results from this trial will contribute to evidence towards constructing a shorter, effective and safe regimen for TB patients. The results will be shared widely with the National Programme managers, policymakers, and stakeholders through open access publications, dissemination meetings, conference abstracts and policy briefs. This is expected to provide a new standard of care for drug-sensitive pulmonary TB patients who will not only reduce the number

of clinic visits and lost to follow-up of patients from treatment but also reduce the burden on the health care system.

Trial Registration: The protocol has been registered on Clinical Trial Registry of India with identifier CTRI/2018/01/011176 on January 8th, 2018.

Strength and Limitations:

- Study design, randomized controlled clinical trial, will add considerable power to the study results and conclusion by decreasing bias (selection bias & observer bias) and minimizing confounding of unequal distribution.
- This study design of randomized controlled clinical trial with stratification will also control of group allocation, enhancing similarity of baseline features including disease severity in the two study arms. This will help us in subgroup analysis improving usefulness for clinical practice
- Due to the strict exclusion and inclusion criteria of the clinical trial, the results from the clinical trial may not be generalizable to all groups of TB patients.
- Due to the multicentric nature of the study, the heterogeneity of data may be present
- Relapse rates may not be known in this trial as the study duration is only for six months of treatment as per the study objective

Keywords: Metformin, Pulmonary Tuberculosis, Clinical trial, Short-course therapy, TB treatment

Introduction:

Globally in 2016, there were an estimated 10.4 million new cases of tuberculosis (TB) with five countries, India, Indonesia, China, Philippines, and Pakistan accounting for 56% of the total cases [1]. There were an estimated 1.3 million TB deaths in 2016 among HIV-negative people and an additional 374000 deaths among people living with HIV [1]. Though effective regimens are available for the treatment of drug-sensitive TB with more than 95% cure, the long duration of such regimens has posed problems for TB treatment and control. This, along with drug toxicity results in poor adherence to treatment resulting in the emergence of drug resistance.

All these have led to an urgent need for more efficient anti-tb drugs, regimens as well as for newer modalities of treating TB. Drugs targeting the TB bacilli can result in the emergence of drug tolerance and resistance, thereby worsening the overall treatment outcomes. Thus there exists a need to consider alternate modalities such as enhancing the host immune system for a faster and complete elimination of the TB bacilli [2, 3]. An efficient and functional immune system is essential to restrain and curb the growth of TB bacilli in the host. Yet, the TB bacilli can still elude the host immune responses, infect the host cells and either multiply or maintain long-term latency in those cells [4, 5]. "Host-targeted" adjunct therapeutic strategies not only augment protective host immune responses but also reduce the chance of development of microbial resistance.

One among the host cell innate antimicrobial arsenal includes the capacity to destroy intracellular pathogens using the phagosomal machinery or autophagy pathway. Intracellular pathogens are effectively controlled by autophagy that is regulated by adenosine monophosphate-activated protein kinase (AMPK) [6, 7]. The *M.tb* virulence results from perturbations in the autophagy network and AMPK signaling [8]. The antidiabetic drug

Metformin (MET; 1, 1-dimethyl biguanide), is an AMPK modulator that inhibits the intracellular growth of *M.tb*, restricts disease immunopathology, and enhances the efficacy of conventional anti-TB drug [9]. Given these promising findings, we plan to test whether the existing approved anti-diabetic drug, Metformin added to the existing anti-TB regimen, with its defined effects on host cell functions could be repurposed for effective and faster treatment of TB as compared to the current standard of care anti-TB regimens.

The Primary objective of this study– the METRIF study- is to study the antibacterial activity, in terms of time to sputum culture conversion of metformin-containing anti-tuberculosis treatment (ATT) regimen instituted during the initial 8-weeks of treatment in patients with newly diagnosed sputum smear-positive pulmonary TB. Secondary objectives of the study are:

i. to compare the time to detection (TTD) of *M.tb* in culture in the group receiving metformin-containing regimen with the control group receiving ATT alone.

ii. To study the autophagy enhancing effect and host immune responses in the two groups,

iii. To examine the post-dosing serum concentration of anti-TB drugs and metformin, their interactions and the impact of genomics - on these parameters (pharmacokinetics (PK) and pharmacogenomics) and

iv. To evaluate the safety and tolerability of metformin by measuring the incidence of Treatment-Emergent Adverse Events.

Methods and Analysis

Study Design and oversight: METRIF is a multi-site, randomized, open-labeled, parallel arm, controlled clinical trial comparing the time to sputum culture conversion among patients with pulmonary TB receiving ATT with metformin (experimental arm) compared to those receiving ATT alone (control arm). The study is randomizing 316 participants to one of the two treatment arms in a 1:1 allocation. The study is sponsored by the India TB Research Consortium of the Indian Council of Medical Research and Open Source Pharma Foundation and implemented by the National Institute of Research in Tuberculosis (NIRT), together with other specialized institutes. The Institutional ethics committee of NIRT has approved the study (NIRT-IEC ID: 2017030, dated 14th December 2017) and National AIDS Research Institute (NARI) (NARI EC / 2018-10 dated 16th February 2018) and will begin enrollment tentatively by 15th June 2018.

Study setting: We will implement METRIF study at three sites in India - NIRT, Chennai and its satellite centers in Madurai and Vellore; All India Institute of Medical Sciences, New Delhi, and, NARI, Pune. These sites will recruit study participants from academic institutions/hospitals as well as community clinics.

Study patients and eligibility: Adult patients, previously untreated and newly diagnosed as pulmonary TB with at least two sputum smear sample, collected on two different occasions, positive for acid-fast bacilli and susceptible to rifampicin detected by cartridge-based nucleic acid amplification test will be eligible for the study. Table 1 provides the detailed inclusion and exclusion criteria. Patients who meet these criteria at presentation and attending the identified study sites will be approached to participate in the study.

Table 1: Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
age 18 years and above	Is pregnant or breastfeeding
Is willing to undergo HIV testing	Has extra-pulmonary TB or drug-resistant TB
Has body weight between 30kg - 65kg	Has body weight < 30 kgs or > 65 kgs
Patient has never received treatment with multidrug anti-TB therapy for more than a week.	Has a prior history of exposure to anti-TB treatment for more than a week
Is willing to use an effective contraceptive method during the study period	Has a history of liver disease or current amino alanine transferase greater than three times the upper limit of normal or total bilirubin concentration greater than 2.5 times the ULN
Is willing to attend a treatment center for supervised treatment and remain within the study area limit	Is serology positive for hepatitis B virus surface antigen or hepatitis C virus antibody
Is willing to sign the informed consent form and adhere to trial procedures and follow-up	Has concomitant psychiatric illness or seizures
Consents for Home visits by the study team	Has concomitant diabetes mellitus or random blood sugar > 200mg or fasting blood sugar >140 mg/dl
	Has serum creatinine >1.2 mg/dl or blood urea >43 mg/dl

Study regimen and Drug dosing: We will randomly assign eligible patients who have provided written informed consent to one of the study regimens in a ratio of 1:1. The site Principal Investigator or his/her nominee who is capable of answering all the trial-related questions from the participants will obtain the study consent. Study participation will last six months: during the first two months, participants will receive the randomly assigned regimen of either daily ATT with metformin or only ATT.

1) Test regimen – Metformin + Isoniazid + Rifampicin+ Pyrazinamide + Ethambutol daily [$2MetHREZ_7$] or

2) Control regimen – Isoniazid+ Rifampicin + Pyrazinamide + Ethambutol daily [$2HRZE_7$]

After 2-months, all study participants in both the arms will receive the standard 4-month continuation phase of HRE daily. Treatment will be supervised and directly observed at a health facility or by directly observed therapy (DOT) provider. Metformin will be dosed at 500 mg once daily for the 1st week and then 1000mg once daily for the remaining period of 7 weeks. Dosing of the other anti-TB drugs will be based on weight bands as shown in table 2.

We will centrally procure all drugs (H, R, E, Z, and Met) to be used in the trial, check for their stated content by validated methods using High-Performance Liquid Chromatography at NIRT Clinical pharmacology department, before dispatching it to the enrolling sites for administration to patients.

Table 2: Weight-based dosing of anti-tuberculosis drugs

Weight Band	Isoniazid	Rifampicin	Pyrazinamide	Ethambutol
30-39 kg	150 mg	300 mg	800 mg	550 mg
40-54 kg	225 mg	450 mg	1200 mg	825 mg
55-65 kg	300 mg	600 mg	1600 mg	1100 mg

Treatment allocation: Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities in chest x-ray and highest sputum smear grading at baseline (<2 or ≥2). Separate randomization lists for each combination of strata for each site will be prepared in advance by an independent statistician, using varying block sizes. Allocation codes will be generated at the central location and at the time of patient's admission to the study, the primary statistician through e-mail will inform the site the regimen based on appropriate stratification factors.

Recruitment process: We will screen all newly diagnosed sputum smear-positive pulmonary TB patients attending the chest clinics at the study sites for study eligibility. Table 3 details the various study procedures. At their first visit, the study will be explained, including the potential risks and benefits associated with participation. We will obtain informed written consent before any protocol-specific screening procedures are carried out. Consenting participants will undergo sputum testing for smear, culture (both solid and liquid media) and rifampicin resistance testing by GeneXpert. Blood samples will be obtained for HIV antibodies (unless the patient is already known to be HIV positive), liver and renal function tests and blood sugar levels. Patients will be re-assessed for eligibility when returning with their investigation results. Those patients who do not have rifampicin resistance and are willing to take part in the study will sign an enrolment consent form (or a thumb-print in the presence of a witness, if illiterate), and randomized to one of the study regimens. During the first two months of treatment, all patients will undergo weekly sputum testing for *M.tb* by smear, liquid, and solid cultures and sparse pharmacokinetics of ATT drugs and metformin. A subset of patients will undergo intense pharmacokinetic study. Randomized patients have an additional blood investigation for immunological and autophagy biomarkers (T-cell, monocyte & dendritic cell

functions both ex-vivo and following stimulation with TB antigens including PPD and ESAT-6/CFP-10, estimation of CRP, TNF-α, and other cytokines) pre and post metformin containing ATT.

We will refer patients ineligible or unwilling to participate in the study to the national programme for treatment as per the existing guidelines.

Table 3: Study schedule of enrolment, interventions, and assessments.

		STUDY PERIOD														
		ENROLMENT	ALLOCATION	INTENSIVE PHASE OF ATT (TRIAL PERIOD)								CONTINUATION PHASE OF ATT (POST-TRIAL PERIOD)				CLOSE OUT
I	ENROLMENT	Baseline (-D7)	W 0	W 1	W 2	W 3	W 4	W 5	W 6	W 7	W 8	M 3	M 4	M 5	M 6	M 7
1	Informed consent	x														
2	Eligibility Screen (including lab tests)	x														
3	Sputum Gene Xpert	x														
4	HIV, Hepatitis B & C	x														
II.	ALLOCATION		x													
III	TRIAL REGIMENS															
	Test Regimen (Metformin containing ATT)		x	x	x	x	x	x	x	x	x					
	Control regimen (Only ATT)			x	x	x	x	x	x	x	x					
	Continuation Phase of ATT (For both groups)											x	x	x	x	
IV	ASSESSMENTS															
1	Clinical Evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2	Sociological Assessment	x	x				x				x	x	x	x	x	x
3	Sputum smear & culture (MGIT, LJ)	x		x	x	x	x	x	x	x	x	x	x	x	x	
4	Urine Pregnancy tests / Sugar / Alb	x					x				x	x	x	x	x	
5	HbA1c	x									x				x	
6	CBC, LFT, RFT, Lactic acid , peripheral smear & chest x-ray	x					x				x				x	
7	Random blood sugar	x			x		x		x		x				x	
8	Pharmacokinetics & genomics						x				x					
9	Immunological studies		x								x				x	
10	Storage Plasma/cell sample		x				x				x				x	
V	OUTCOMES															x

Treatment delivery, compliance, and retention: ATT will be administered under direct observation for 6-days of the week and supplied for the seventh day. Study staff will reinforce the importance of adherence to the treatment schedule at each visit. If the patient misses drug doses in the intensive phase (IP) or continuation phase (CP), it will be compensated at the end of the respective stages over next 15 days, so that the patient receives 60 doses of the assigned regimen in the IP and 120 doses in the CP. Treatment supporters and enablers will facilitate retention of the participants in the study. All treatment is ambulatory and delivered by dedicated DOT provider. We will assess treatment adherence through reviews of treatment card throughout the treatment phase. In case of drug supply for any reason, empty pill cover count will determine adherence. Treatment adherence will be enhanced by – reimbursing the transport costs and loss of wages incurred by the participant during the study visits. During the study period, participants will receive food supplement every month and meals during their extended, study visits.

Concomitant medication while in the trial: Concomitant antibiotic treatments of any kind are discouraged during the study period. If required, their usage is restricted for a short course (< 2 weeks) period. Concomitant non-antibiotic drug usage is allowed in small quantity. Only those considered necessary for the subject's welfare and are unlikely to interfere with the study medication, may be given at the discretion of the investigator. Below are the drugs used to treat *M. tb* infections which should not be used during this trial: Streptomycin, Thiacetazone, PAS, Dapsone, Amoxicillin-clavulanic acid/ clavulanate, Clofazimine, Capreomycin, any oxazolidinone antibiotic (e.g., linezolid), Ofloxacin, Levofloxacin or Moxifloxacin.

Criteria for Discontinuation/ Withdrawal of study participants: Withdrawal of study participants can occur for any of the following reasons – Pregnancy, if the investigators feel that staying in the study is harmful to the patient, if the patient does not follow study procedures or is

not available for appointments, if the study sponsor or NIRT or representative of the Drugs Controller General of India decide to stop or cancel the study, if the Data and Safety Monitoring Board recommends halting the study or if the patient wishes to withdraw for any reasons. In such cases, appropriate treatment, as per the standard of care treatment available then will be ensured for patients taking into consideration the drug susceptibility pattern of the individual.

Study Outcomes: The primary outcome is the time to sputum culture conversion, which is assessed by the time interval between the date of treatment initiation and the date of acquisition of the first of at least two consecutive negative cultures taken at least eight days apart. We will also assess, on a weekly basis, the time to positivity, a change in *M.tb* $\log_{10}$ colony forming units (CFU) in culture and the proportion of participants with sputum culture negativity in the two treatment arms.

The PK, the area under the concentration curve (AUC), will be assessed through blood sampling on a single day during the first month of ATT, after a minimum of seven doses of RMP and MET (1000mg). We will estimate the Minimum inhibitory concentration (MIC) of each participant's pre-treatment infecting isolate from early morning and overnight sputum samples collected at the pre-treatment visit. We will also compare the Genomics results of Metformin and Rifampicin with the plasma concentration of these drugs, bacteriological and clinical endpoints along with the drug-drug interaction of metformin and rifampicin.

For the occurrence of Treatment-Emergent Adverse Events (TEAEs), clinical investigators will grade clinical and laboratory abnormalities according to the modified Adult Toxicity Table for the Division of Microbiology and Infectious Diseases, National Institutes of Health [10]. The safety and tolerability analysis will include all patients who were randomized to and received at least one dose of the study regimen.

Participant Timeline: The trial will consist of three stages - Screening and enrolment (a maximum of 1 week); the Intensive phase of treatment with or without test drug (2 months) followed by a continuation phase of standard drugs (4 months). Table 3 shows the schedule of enrolment, interventions, and assessments of participants in the trial.

Patient and public involvement: Patients were not involved in the development of the research question, or the design of this study as the scientific problem is still not proven and only in the research arena. However, considering the relevance of the study outcome to public health, and policy-makers, the study is discussed in our Institutional Scientific advisory committee, Institutional Ethics committee and Community Advisory Board consisting of a representative from affected community, peers and responsible members of the society. Patients will be involved in the recruitment and conduct of the study. We will disseminate the results of the study widely through meetings, workshops, conference presentation, and publication

Sample Size Assumption: Published literature has shown a median time to sputum culture conversion by a liquid medium with daily ATT to be 32 days [11]. With the addition of metformin, we assumed a 30% reduction in the time to culture conversion, i.e., to approximately 22 days. We estimated that we would require 150 new sputum smear-positive patients to show this difference at 80% power, an alpha level of 0.05 and a hazards ratio of 1.5. With the assumption that 5% of patients will be lost to follow-up or not assessable in the primary analysis, a total target sample size of 158 patients in each treatment arm will be recruited, totaling to 316 patients for the study.

Randomization Procedures: Permuted block Randomization will be done centrally using a computer-generated list of random numbers, stratified by presence or absence of cavities on chest x-ray and highest sputum smear grading (<2 or >2). The randomization sequence will not

be available to those who enroll participants. Allocation codes will be generated at the central site, NIRT and will take place at the time of patient's admission to the study. Upon receiving an electronic-mail request from the study sites, for allocation of a participant to the study, the NIRT statistician will do allocation procedure centrally based on appropriate stratification factors. He/She will then inform the site physician, through e-mail, the study regimen along with the unique study ID for patient enrolment to the study.

Data Collection, Management, and Interim Analysis: Study randomization will be done centrally by the Central Data management (CDM) unit at NIRT, Chennai over e-mail. During the study conduct, data will be collected at sites at baseline and follow-up on pre-defined case record forms (CRF) and transmitted electronically to the CDM unit. Data correctness and completeness will be checked before sending. The central team will also conduct a periodic quality check of the data. Data collected will include a) improvement in disease status regarding abetment of symptoms, signs and sputum cultures conversion and b) safety and tolerability of the regimen concerning both clinical and laboratory adverse events.

During the trial all essential trial documents including the source documents, informed consent forms, etc. will be stored securely under lock and key at the recruiting sites under the supervision of the site investigator. All e-data will be password protected with limited access to the investigator and their teams alone. The study team will retain the records for a minimum period of five years after completion of the study.

The study has two interim analyses planned, viz. after 33% and 66% of the enrolled patients have completed 8-weeks of Metformin treatment and with sputum culture results available for review. The study also has an additional interim analysis for reported serious

adverse events with frequency higher than anticipated. The final analysis will include all enrolled patients when they have completed six months of ATT.

Study Outcome analysis: The primary efficacy analysis will compare the median time to culture negativity. This analysis will happen when the last enrolled patient has completed eight weeks of treatment and will be done using culture results from liquid culture (MGIT) and in those who are not isoniazid or RMP mono-resistant at baseline. Both a Modified Intent to Treat (MITT) and a Per Protocol (PP) analysis will be conducted. Secondary outcome parameters will include proportion and time to sputum culture positivity using the MGIT system. The safety and tolerability analysis will consist of all patients who were randomized to and received at least one dose of the drug. Based on plasma drug concentrations obtained at different time points, we will calculate certain pharmacokinetic variables related to study drugs. Drug peak concentrations (Cmax) and exposure (AUC) will be linked to time to sputum conversion and occurrence of adverse events

Data Safety Monitoring Board (DSMB): The DSMB for this study comprises of TB clinicians, pharmacokinetic specialists, and an independent biostatistician. They will review data from this trial on a regular basis, including incidence progress of the trial, and detect evidence of early safety issues for the trial participants with a specific focus on grade 3 or 4 AEs, SAEs, and treatment discontinuations due to AE. Based on the results, DSMB shall make recommendations on continuing or terminating / modifications to the trial.

Clinical Site Monitoring & Quality assurance: An independent study Monitor, appointed by the sponsor, will be responsible for monitoring data quality by trial Standard Operating Procedures. Based on the monitoring plan, field visit and audit will be performed at different stages. All participant records, CRFs, and other source documents for the patients recruited in

this study will be made available for review by the monitors. A meeting of the investigators of each local site will convene monthly via web-based remote conference system to share the progress of the study and discuss with the problems met during the trial conduct. During the conduct of the research, any critical protocol modifications will be informed to the IEC, trial registry and if relevant to the trial participants.

Confidentially of trial data: The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the antibacterial activity, safety, and tolerability of the investigational product used in this trial. These data will be processed with adequate precautions to ensure confidentiality. Trained professional will collect al study data with the utmost sensitivity and confidentiality. The study participant will be informed during the informed consent process that - the monitor(s), the IEC, and the regulatory authorities will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the participant, to the extent permitted by the applicable laws. Otherwise, only the study investigator and his/her team will have access to the trial data.

Ethics and Dissemination

This trial will proceed as per the current ICH Good Clinical Practice and the ICMR Ethical guidelines for biomedical research in human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial subjects are protected; consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible. The confidentiality of the study participants will be protected throughout the study period as per provision in the Indian-GCP & applicable regulations by laws

of India. The processing of personal data in this trial will be limited to those data that are reasonably necessary to investigate the antibacterial activity, safety, and tolerability of the investigational product used in this trial.

The study principal investigator holds primary responsibility for the preparation of manuscripts and materials for result dissemination and publication. Once the trial is complete, the investigators anticipate publishing results of this study in several peer-reviewed scientific journals, present the abstracts in meetings, to stakeholders and policymakers and share the results widely with the Programme managers. During all these processes, details of the trial participant will remain confidential.

Discussions:

Currently, for the management of new sputum smear-positive drug-sensitive pulmonary TB patients, 6-month regimens are used in more than 90 countries that are evaluated to be effective [12]. Newer anti-TB drugs are also in the pipeline. However, all types of treatment currently available are pathogen-targeted and with toxic drugs. Drug-toxicity can lead to poor treatment adherence resulting in treatment failure and development of resistance. As the pathogen-targeted strategies may lead to the development of acquired microbial resistance, new "host-targeted" adjunct therapeutic approaches not only are less likely to engender microbial resistance but also augment protective host immune responses thus accelerating bacterial clearance from the system.

Metformin has an inhibitory effect on mitochondrial complex I, inhibition of which has been found to increase the AMP/ATP ratio [13, 14]. The altered cellular energy status induces activation of AMPK, a serine/threonine kinase, and acts as an energy sensor [15]. Activation of

AMPK by metformin stimulates endothelial nitric oxide synthase (eNOS) activity which leads to bacterial killing [16]. Metformin also acts through AMPK-independent mechanisms. It promotes phagocytosis, phagolysosome fusion & autophagy in macrophages. Macrophages exposed to metformin had higher bactericidal capacity attributed to increased mitochondrial Reactive Oxidative Species (ROS) production required for bacterial killing [17]. Approximately 90% of the newly diagnosed sputum positive patients are sensitive to isoniazid (H) and rifampicin (R), adding the drug metformin would have a beneficial effect in the early killing of intracellular bacteria by influencing the host immunity. Experiments in mice treated with MET (200 mg/kg) along with H 10mg/kg showed not only a considerable reduction in the bacillary load in the lungs but also reduced areas of lung tissue damage compared to H-alone treatment [9]. This dose of MET is equivalent to approximately 1200 mg/day for a 60-kg human and this dose of MET will be used in this trial along with H, R, E and Z in the intensive phase of daily ATT. High dose of metformin has also been used in clinical practice for the management of diabetic individuals with and without TB and in non-diabetic conditions like polycystic ovarian syndrome and obesity [18, 19]. Though the WHO recommends 2HRZE/4HR regimen for new patients with PTB, given the high prevalence of Isoniazid resistance in the country (11% in new sputum positive patients),the Revised National TB control programme of India recommends using three drugs in the continuation phase i.e. 2HRZE/4HRE regimen which will be followed in this trial [20].

We are proposing a Phase IIB trial looking at sputum-culture conversion to negative over a 2-month period as studies have shown a correlation between positive 2-month sputum-culture status and subsequent relapse. However, limitations of this design include the binary outcome of 2-month sputum-culture endpoint, hence requiring larger samples size to prove the benefit. To

overcome this limitation, we plan serial sputum colony counting (SSCC) and time-to-detection (TTD). SSCC involves counting of viable mycobacterium tuberculosis bacilli at various time points during the 8-week period. This measurement will demonstrate the rapid bacillary killing in the early phase and track the rate and pattern of culture conversion through-out the 8-weeks, thereby providing a better marker for culture conversion [21]. However, TTD in liquid culture is considered an even better choice than SSCC as the complications of counting viable colonies is replaced by automated measurement of detection time of the bacilli through-out the 8-week period. In our study, as the outcome measures of interest (primarily bacteriological) are based on objective microbiology, the bacteriology laboratory staff will be blinded to the patient's treatment. Safety assessments will be defined as objectively as possible, using pre-defined grading criteria for laboratory abnormalities and adverse events. The pharmacokinetic aspect of the study is based on a population approach which is facilitated by the intensive-sparse sampling design. The study will estimate the pharmacokinetic exposure (AUC0–24) for all study participants. Several alternative biomarkers of treatment response are also being evaluated using these samples.

Results from METRIF study will complement observations from other retrospective and case-control studies that showed metformin to be a protective agent against TB infection among people with diabetes [22, 23]. As studies did not show any dose-dependent protection in metformin users for TB, we will be using 500mg dose for the first week followed by 1000mg for the remaining 7-weeks. This dose escalation is being done to reduce the gastrointestinal side effects of metformin. If the study regimen is successful, it will pave the way to evaluate shorter regiments for the treatment of PTB. This trial will establish a new standard of care for DS-PTB that will not only reduce the number of required clinic visits by the patients but also decrease the

proportion of patients who fail to complete the full course of therapy. It should also reduce the overall burden on the health care system. If the study regimens are shown to be superior or at least non-inferior to the control regimen, then the procedure represents an even greater advantage for both patients and TB control programmes throughout the world.

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- ***Authors' contributions***: CPP, SS, RG & TB – conceived & designed the study / CPP, PKB, MN, CPR, HK, NSG, MSJ, MS, & SS – development, and writing of the study protocol and this manuscript
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- ***Competing interests***: None

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	15
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6 & 7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12& 20

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___13_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	____n/a_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____13_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____12_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_13 & 14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____n/a_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____6_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____14_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Yes
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Yes

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.