

Safety and Tolerability of Metformin in People with Tuberculosis (TB)
and Human Immunodeficiency Virus (HIV) (METHOD)

NCT04930744

METHOD Trial Protocol Version 15.0

Document Date: 19 August 2024

Title: A Prospective, Randomized Open-Label Phase II Study of the Safety and Tolerability of Metformin in Combination with Standard Antimicrobial Treatment of Pulmonary Tuberculosis in People Co-infected with HIV

Acronym: METHOD Trial

Clinical Trial Phase: 2A

Local Protocol Number: AUR1-1-199

DAIDS-ES Document Number: 38739

NIAID Funding Mechanism: 1U01AI134585-01A1

Principal Investigators:

Hardy Kornfeld, MD (University of Massachusetts Medical School); Amit Singhal, PhD (Singapore Immunology Network); Robert S. Wallis, MD (The Aurum Institute)

Co-Investigators:

Wenjun Li, PhD (University of Massachusetts Lowell); Gavin Churchyard, MBBCh, PhD (The Aurum Institute); Gary Maartens, MBChB, MMed, FCP SA DTM&H (University of Cape Town)

IND Sponsor: The Aurum Institute

IND Number: 153703

Protocol Chair/Co-Chair:

Chair: Robert Wallis; Co-Chair: Hardy Kornfeld

DAIDS Medical Officer:

Melanie Goth, MD, FAAP

Version Number: 15.0

Version date: 19 August 2024

1 SIGNATURE PAGE

I agree to conduct the study in accordance with the relevant, current protocol and will not make changes to the protocol without permission of DAIDS, except when necessary to protect the safety, rights, or welfare of study participants.

I agree to personally conduct or supervise this study.

I will ensure that the requirements relating to obtaining informed consent and Ethics Committee (EC) or Institutional Review Board (IRB) review and approval (insert relevant terms of assurance here, e.g. 45 CFR 46, ICH/GCP, etc.) are met.

I agree to report to the sponsor adverse experiences as per that occur during the course of this study.

I agree to maintain adequate and accurate study records and to make those records available for inspection by DAIDS, DAIDS' authorized representatives, and/or other applicable regulatory entities.

I will ensure that an EC or IRB that complies with the requirements of 45 CFR Part 46 will complete initial and continuing review and approval of the study. I also agree to promptly report to the EC/IRB all changes to the study and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes to the study without DAIDS and EC/IRB approval, except where necessary to eliminate apparent immediate hazards to study participants.

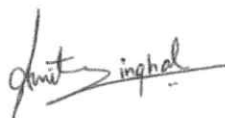
I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigators:

Hardy Kornfeld, MD; Amit Singhal, PhD; Robert S. Wallis, MD

 09 November 2024

Signed (Date/Name/Title)

 9th Nov 2024

 11 Nov 2024

2 TABLE OF CONTENTS

1	SIGNATURE PAGE	2
2	TABLE OF CONTENTS	3
3	KEY ROLES	6
4	LIST OF ABBREVIATIONS	8
5	PROTOCOL SUMMARY	11
6	INTRODUCTION	14
6.1	Background Information	14
6.2	Rationale	19
6.3	Study Hypotheses	21
7	OBJECTIVES	22
7.1	Primary Objective(s)	22
7.2	Secondary Objective(s)	22
7.3	Exploratory Objective(s)	22
8	STUDY DESIGN	23
9	STUDY POPULATION	25
9.1	Inclusion/Exclusion Criteria	25
9.1.1	Participant Inclusion Criteria	25
9.1.2	Participant Exclusion Criteria	25
9.1.3	Co-enrollment Criteria	26
9.2	Recruitment Process	26
9.2.1	Potential candidates for Participation in the Treatment Arms	26
9.2.2	Potential candidates for Participation in the Omics Control Group	27
9.3	Participant Retention	27
10	INTERVENTIONS	28
10.1	Biomedical Interventions	28
10.1.1	Regimen (dose, schedule, route, administration)	28
10.1.2	Study Product Formulation and Preparation	28
10.1.3	Study Product Supply and Accountability	28
10.1.4	Assessment of Participant Adherence with Study Product	28
10.1.5	Concomitant Medications and Procedures	29
10.1.6	Permitted Medications and Procedures	29
10.1.7	Prohibited Medications and Procedures	29
10.1.8	Precautionary Medications and Procedures	29
10.1.9	Required Medications	30
10.1.10	Rescue Medications	30
10.2	Behavioral Intervention(s)	30
11	STUDY PROCEDURES/EVALUATIONS	31
11.1	Clinical Evaluations and Procedures	31
11.1.1	Medical history	31
11.1.2	Medication history	31
11.1.3	Physical exam	32
11.1.4	Counselling procedures	32
11.1.5	Radiological procedures	32
11.1.6	Questionnaires	32
11.1.7	Electrocardiogram	32
11.1.8	Lung health assessment	32
11.2	Laboratory Evaluations	33
11.2.1	Clinical Laboratory Services	33
11.2.2	Clinical chemistries	33

11.2.3	Hematology, Immunology and Virology.....	34
11.2.4	Microbiology.....	35
11.2.5	Whole Blood Bactericidal Activity.....	35
11.2.6	Pharmacokinetics	36
11.2.7	Research Samples.....	37
11.2.8	Specimen Preparation, Handling and Shipping	37
11.2.9	Biohazard Containment	37
11.2.10	Total Blood Volume	37
11.3	Schedule of Procedures/Evaluations: Timing and Definitions	38
11.3.1	Screening	38
11.3.2	Enrollment	39
11.3.3	HIV Counselling and Testing.....	39
11.3.4	Follow-Up	39
11.3.5	Early Termination Visit	42
11.3.6	Pregnancy	43
11.3.7	Other Visits	44
11.3.8	Final Scheduled Follow-up Clinic Visit.....	45
11.3.9	Final AE Assessment.....	45
12	ASSESSMENT OF SAFETY	46
12.1	Safety Assessment Overview.....	46
12.2	Adverse Event Procedures and Reporting Requirements.....	48
12.2.1	General Considerations	48
12.2.2	Expedited Reporting of Adverse Events Using the DAIDS Adverse Experience Reporting System (DAERS)	49
12.2.3	Reporting Requirements for the METHOD trial	49
13	CLINICAL MANAGEMENT	50
13.1	Clinical Management of Adverse Events.....	50
13.1.1	GI Toxicity.....	50
13.1.2	Hyperlactatemia and Lactic Acidosis	51
13.1.3	Reproductive Risks.....	51
13.1.4	Other Toxicities.....	52
13.2	Other Disease Events	52
13.3	Pregnancy	53
13.4	Breastfeeding/Replacement Feeding	53
13.5	Treatment Failure.....	53
13.6	Criteria for Discontinuation	54
13.6.1	Criteria for Permanent Intervention Discontinuation for an Individual Participant..	54
13.6.2	Criteria for Premature Study Discontinuation for an Individual Participant.....	54
14	STATISTICAL CONSIDERATIONS	56
14.1	Overview and General Design Issues	56
14.2	Study Endpoints.....	56
14.2.1	Primary Endpoint	56
14.2.2	Secondary Endpoint(s).....	56
14.2.3	Exploratory Endpoint(s).....	57
14.3	Study Objectives and Hypothesis/Hypotheses	57
14.4	Sample Size Considerations	59
14.5	Enrollment/Stratification/Randomization Procedures/Unblinding Procedures	61
14.6	Participant Enrollment and Follow-up	62
15	DATA AND SAFETY MONITORING	63
15.1	Planned Interim Analyses and Stopping Guidelines	63

15.1.1	Interim Safety Review	63
15.1.2	Interim Efficacy Review	64
15.2	Analysis Plan	64
16	DATA HANDLING AND RECORDKEEPING	68
16.1	Data Management Responsibilities	68
16.2	Essential/Source Documents and Access to Source Data/Documents	69
16.3	Quality Control and Quality Assurance	69
17	CLINICAL SITE MONITORING	70
17.1	Project Management	70
17.2	Clinical Monitoring and Site Management	70
17.3	Systematic Off-Site Document and Data Review and Analysis	71
17.4	On-Site Monitoring	71
17.5	Sponsor Audits	71
18	HUMAN SUBJECTS PROTECTIONS	72
18.1	Ethics Committee/Institutional Review Board	72
18.2	Vulnerable Participants	74
18.2.1	Pregnant women and fetuses	74
18.2.2	Prisoners	74
18.2.3	Children	74
18.2.4	Illiterate participants	75
18.3	Informed Consent	75
18.3.1	Informed Consent Process	75
18.3.2	Documentation of Informed Consent	75
18.3.3	Waiver of Informed Consent	76
18.3.4	Waiver of Documentation of Informed Consent	76
18.3.5	Stored Samples and Associated Data Considerations	76
18.4	Risks	76
18.5	Social Impact Events	77
18.6	Benefits	77
18.7	Compensation	78
18.8	Participant Privacy and Confidentiality	78
18.9	Certificates of Confidentiality	78
18.10	Critical Event Reporting	78
18.11	Communicable Disease Reporting	81
18.12	Incidental Findings	81
18.13	New Findings	82
18.14	Study Discontinuation	82
18.15	Post-Trial Access	82
18.16	Ancillary Benefits	82
18.17	Community Advisory Group and Other Stakeholders	82
19	ADMINISTRATIVE PROCEDURES	84
19.1	Protocol Registration	84
19.2	Regulatory Oversight	86
19.3	Study Implementation	86
19.4	ClinicalTrials.gov	87
20	PUBLICATION POLICY	88
21	REFERENCES	89
22	APPENDICES	98

3 KEY ROLES

Robert S. Wallis, MD (PI/PD and Protocol Chair)

The Aurum Institute, 29 Queens Rd, Parktown, Johannesburg 2193, South Africa; +27 (0)74-609-2779; +1 860-271-6745; rwallis@auruminstitute.org

Hardy Kornfeld, MD (PI/PD and Protocol Co-Chair)

University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655 USA; +1 508-856-2646; Hardy.Kornfeld@umassmed.edu

Amit Singhal, PhD (PI/PD)

A*STAR Infectious Diseases (ID) Labs, 8A Biomedical Grove, Immunos Building, Singapore 138648; +65 6407-0631; amit_singhal@idlabs.a-star.edu.sg

Wenjun Li, PhD (Co-I, Chief Statistician)

University of Massachusetts Lowell, 61 Wilder St, Lowell, MA 01854 01655 USA; +1 978-934-4515; Wenjun_Li@uml.edu

Gavin Churchyard, MBBCh, PhD (Co-I)

The Aurum Institute, 29 Queens Rd, Parktown, Johannesburg 2193, South Africa; +27 (0) 82-338-3557; gchurchyard@Auruminstitute.org

Gary Maartens, MBChB, MMed (Co-I, Lead Pharmacologist)

University of Cape Town Health Sciences, Division of Clinical Pharmacology, Anzio Road, Observatory 7925, Cape Town, South Africa; +27 (0) 21-406-6008; gary.maartens@uct.ac.za

Nicole Glover MBBCh Site PI)

The Aurum Institute Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive View, Tembisa, Gauteng, South Africa, 1632, +27 72 277 2261; nglover@auruminstitute.org

Kinita Naidoo MBBCh, (Site PI)

Isango Lethemba TB Research Unit, A Division of the Wits Health Consortium, Jose Pearson TB Hospital, Mission Road, Bethelsdorp, Gqeberha, 6003, 079 656 9118; KiNaidoo@witshealth.co.za

Pholo Maenetje, PhD (Scientist)

The Aurum Institute Tembisa Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive, Hospital View, Tembisa, Gauteng, South Africa, 1632; +27 (0) 10 590 1561; pmaenetje@auruminstitute.org

Matsie Lydia Mngomezulu (Project Manager)

The Aurum Institute Tembisa Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive, Hospital View, Tembisa, Gauteng, South Africa, 1632; +27 87 135 1659; lmngomezulu@auruminstitute.org

Carita Marx (Pharmacy Manager)

The Aurum Institute Tembisa Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive, Hospital View, Tembisa, Gauteng, South Africa, 1632; +27 (0) 10 590 1654; CMarx@auruminstitute.org

John Mdluli (HOD Community)

The Aurum Institute, Tembisa Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive, Hospital View, Tembisa, Gauteng, South Africa, 1632; +27 (0) 10 590 1654; jmdluli@Auruminstitute.org

Themba Phakathi (Data Management)

The Aurum Institute, 29 Queens Road, Parktown, Johannesburg, South Africa; +27 (0) 10 590 1661; tphakathi@auruminstitute.org

Mandla Mlotshwa, PhD (Laboratory Manager)

The Aurum Institute Tembisa Clinical Research Centre, Cnr Rev RTJ Namane and Flint Mazibuko Drive, Hospital View, Tembisa, Gauteng, South Africa, 1632; +27 (0) 10 590 1785; MMlotshwa@auruminstitute.org

Stacey Lawrence (Regulatory Manager)

The Aurum Institute, 29 Queens Road, Parktown, Johannesburg, South Africa; +27 (0) 10-590-1369; regulatory@auruminstitute.org

Aruna Bhoola (Regulatory Manager)

The Aurum Institute, 29 Queens Road, Parktown, Johannesburg, South Africa; +27 (0) 10-590-1369; regulatory@auruminstitute.org

Robert N. Mahon, PhD (DAIDS Program Officer)

Tuberculosis Clinical Research Branch, NIH/NIAID, 5601 Fishers Lane, Rockville, MD 20852; +1 (240) 669-3062; robert.mahon@nih.gov

Melanie Goth, MD, FAAP (DAIDS Medical Officer)

Tuberculosis Clinical Research Branch, NIH/NIAID, 5601 Fishers Lane, Rockville, MD 20852; +1 (301) 761-7490; melanie.goth@NIH.gov

4 LIST OF ABBREVIATIONS

AE	Adverse Event
AMPK	AMP Kinase
ART	Antiretroviral Therapy
ATT	Anti-Tuberculosis Treatment
BMI	Body Mass Index
CAB	Community Advisory Board
CAG	Community Advisory Group
CE	Critical Event
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
COR	Contracting Officer's Representative
CRA	Clinical Research Associate
CRC	Clinical Research Centre
CRF	Case Report Form
CRO	Contract Research Organization
CQMP	Clinical Quality Management Plan
CYP	Cytochrome P450
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DM	Diabetes Mellitus
DTG	Dolutegravir
DAIDS PRO	DAIDS Protocol Registration Office
DPRS	DAIDS Protocol Registration System
DSMB	Data Safety Monitoring Board
E	Ethambutol
EAE	Expedited Adverse Event
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
EFV	Efavirenz
EQA	External Quality Assurance
FBG:	Fasting Blood Glucose
FDA	U.S. Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FSBG	Fingerstick blood glucose
FTC	Emtricitabine
FWA	Federalwide Assurance
GCP	Good Clinical Practices
GCLP	Good Clinical Laboratory Practices
GDS	Genomic Data Sharing
GI	Gastrointestinal
HFP	Hepatic function panel
H	Isoniazid
HBsAg	Hepatitis B Surface Antigen

HDT	Host-Directed Therapy
HHS	U.S. Department of Health and Human Services
HIV	Human Immunodeficiency Virus
IC	Informed Consent
ICF	Informed Consent Form
ICH	International Council for Harmonization
IF	Incidental Finding
IND	Investigational New Drug Application
IoR	Investigator of Record
IPT	Isoniazid Preventative Therapy
IR	Immediate Release
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LA	Lactic Acidosis
LoA	Letter of Amendment
MALA	Metformin-Associated Lactic Acidosis
MGIT	Mycobacterial Growth Indicator Tube
MO	Medical Officer
Mtb	Mycobacterium tuberculosis
NHLS	National Health Laboratory Service
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NTRDA	National Tuberculosis and Respiratory Disease Association
OHRP	U.S. Office for Human Research Protections
OSCO	Office for Clinical Site Oversight
PBMC	Peripheral Blood Mononuclear Cells
PD	Pharmacodynamic
PI	Principal Investigator
PIAT	Pulmonary Impairment After Tuberculosis
PK	Pharmacokinetic
PM	Project Manager
POC	Point of Care
PR	Protocol Registration
PRO	Protocol Registration Office
R (RIF)	Rifampicin
SAE	Serious Adverse Event
SAHPRA	South African Health Products Regulatory Authority
SC	Subcutaneous
SGRQ	St. George's Respiratory Questionnaire
SH	Symptomatic hyperlactatemia
SIgN	Singapore Immunology Network
SOP	Standard Operating Procedure
SRC	Scientific Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TB-IRIS	Immune Reconstitution Inflammatory Syndrome

TDF	Tenofovir disoproxil fumarate
TMF	Trial Master File
UCT	University of Cape Town
ULN	Upper Limit of Normal
UMMS	University of Massachusetts Medical School
UP	Unanticipated Problem
WBA	Whole Blood Bactericidal Activity
WHREC	University of Witwatersrand Human Research Ethics Committee
WHO	World Health Organization
WITS	University of the Witwatersrand
XR	Extended release
Z	Pyrazinamide
6MWT	6 Minute Walk Test

5 PROTOCOL SUMMARY

Full Title

A Prospective, Randomized, Open-Label Phase II Study of the Safety and Tolerability of Metformin in Combination with Standard Antimicrobial Treatment for Pulmonary Tuberculosis in People Co-infected with HIV

Short Title

METHOD TRIAL

Study Sample Size

Total cohort sample size N=112, comprising 56 participants each in two parallel study arms (standard therapy or standard therapy plus metformin) with the goal of retaining 100 participants with evaluable data for analysis. An additional 30 HIV⁺ persons without active TB disease will be recruited as controls for a single visit to provide baseline values for proteomic, transcriptomic and flow cytometry studies. The total cohort including control participants and participants enrolled into the longitudinal treatment study comprises 142 individuals.

Study Population

HIV-positive adults (treated or ART-naïve) newly diagnosed with sputum culture-positive, drug-sensitive pulmonary tuberculosis (TB). The systems biology “omics” control group will include those (treated or ART-naïve) without evidence of active TB.

Participating Sites

Clinical site 1:

The Aurum Institute Tembisa Clinical Research Centre (CRC), Gauteng, South Africa

Satellite Site 1:

The Aurum Institute Pretoria Clinical Research Centre (CRC), Pretoria, Gauteng, South Africa (satellite for intensive PK sampling)

Clinical site 2:

Isango Lethemba TB Research Unit, Port Elizabeth, South Africa

Other collaborating sites:

University of Massachusetts Medical School, Worcester, MA, USA.

University of Massachusetts Lowell, Lowell, MA USA.

A*Star, Infectious Diseases (ID) Labs, Singapore.

University of Cape Town, Cape Town, South Africa.

Study Design

Phase 2A randomized, open-label trial of metformin added to standard anti-tuberculosis treatment (ATT) and anti-retroviral therapy (ART) in TB/HIV co-infected patients.

Study Schema

See Appendix A, Figure 1, for the interventional study schema. See Appendix A, Figure 2, for the omics control group study schema.

Schedule of Procedures/Evaluations

See Appendix B, Table 1, for the interventional study schedule of evaluations. See Appendix B, Table 2 for the omics control group schedule of evaluations.

Study Duration

The duration of the METHOD trial is 5 years. The duration of individual participation in the interventional arms of the study will be 36 weeks, not including an initial period of screening with an interval of up to 14 additional days prior to study enrollment. All participants in the interventional study will take standard ATT for drug-sensitive pulmonary TB starting at enrollment. Participants in the metformin arm will begin taking metformin 1 week later and metformin will be stopped on week-12. The final scheduled clinic visit coincides with the completion of ATT at week-24 and the final follow-up contact is a phone interview at week-36.

Omics control group participants will have a screening visit and (if eligible) an enrollment visit within a period of 2 to 7 days, depending on scheduling.

Study Regimen/Intervention

Metformin hydrochloride 500 mg tablets added to standard ATT for drug-sensitive TB. Metformin is initiated as one 500 mg tablet PO daily, beginning one week after starting ATT. One week later (study week-2), the dose is increased to one 500 mg tablet PO twice daily and that dose is continued until study week-12.

There is no experimental drug regimen for the standard treatment arm of the interventional study or for participants in the omics control group.

Primary Objectives for the Interventional Study

1. Determine tolerability and safety of metformin added to standard ATT and ART in the treatment of TB/HIV co-infection.
2. Conduct a pharmacokinetic-pharmacodynamic analysis of metformin toxicity, efficacy, and immune modulation.

Primary Objective for the Omics Control Group

To provide background values for proteomic, transcriptomic and flow cytometric analyses in otherwise healthy HIV+ adults without active TB disease for comparison to the two treatment arms of the METHOD trial.

Primary Endpoint

The number of grade 3 or higher events during the time on study drug plus 4 weeks post study drug completion.

There is no primary clinical endpoint to follow for the omics control group.

Secondary Objective

To evaluate the efficacy of metformin as adjunctive therapy when added to standard ATT and ART for TB/HIV co-infection. Efficacy will be determined based on the parameters listed as secondary endpoints.

Secondary Endpoints

1. Time from treatment initiation to the first of two consecutive negative once-weekly sputum cultures for *Mycobacterium tuberculosis* (Mtb) without an intervening positive culture in liquid media, up to 12 weeks.
2. Change in radiographic severity score between baseline and study completion.
3. Change in percent predicted FEV1 (FEV1%) between baseline and study completion.
4. Change in 6-minute walk test distance-saturation product between baseline and study completion.

-
5. Change in St. George's Respiratory Questionnaire (SGRQ) score between baseline and study completion.

6 INTRODUCTION

6.1 Background Information

Tuberculosis (TB) remains a leading cause of death from infection worldwide and the leading of death in people co-infected with HIV ¹ despite the availability of effective antibiotics since the 1950s. The requirement of prolonged, multi-drug regimens for cure is a major barrier to TB elimination. The current standard ATT regimen for drug-sensitive TB is a 6 month “short” course of rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E): 2(RHZE)/4(RH). Results of treatment shortening trials have been disappointing. A trial reported by Johnson et al. in 2009 ² enrolled patients with non-cavitary disease on entry and negative cultures after 8 weeks of standard treatment, a population thought to be at low risk of recurrence, and randomly assigned them to a total of 4 or 6-months ATT. Although rates of recurrence were indeed low in both arms, enrollment was terminated early, as the study’s hypothesis – that treatment duration would not affect relapse risk – was proven incorrect. More recently, the RIFAQUIN, REMox and OFLUTUB trials tested treatment shortening with different fluoroquinolone-containing regimens ³⁻⁵. All three failed to show noninferiority of 4-month regimens compared to standard 6-month ATT. These results indicate that a new paradigm is required to make more than incremental steps towards TB treatment shortening.

Pulmonary impairment after TB (PIAT) occurs in ~50% of patients who complete curative antimicrobial treatment ⁶. A study by Willcox et al. in Cape Town found the extent of permanent lung impairment directly related to the radiographic extent of disease at diagnosis ⁷. Patients presenting with moderately advanced disease according to National Tuberculosis and Respiratory Disease Association (NTRDA) criteria had a 30% risk of moderate lung impairment (FEV1 50-65% of predicted). Those presenting with far advanced disease had a similar risk of moderate impairment, plus an additional 30% risk of severe impairment (FEV1 <50% of predicted). Lung damage of this extent will have profound effects on survival and disability adjusted life years. Indeed, one study of 3,933 US TB patients deemed “cured” found 22% had died in the following 11 years, shortening their expected longevity by a mean of 3.6 years vs controls ⁸. A similar study of 3,201 Israeli patients found their standardized mortality risk to be 3.7 times that of the general population ⁹. The authors found 12% of former TB patients had died in the subsequent 11 years; a substantial proportion of these deaths were attributable to the chronic effects of lung damage caused by TB. Given the high risk and lifelong morbidity of PIAT, there is a clear rationale to investigate the potential for therapies designed to preserve lung structure and function during TB treatment.

The need for faster and more effective TB treatment is most acute in South Africa, which is home to 0.7% of the world’s population, but has 17% of the global burden of HIV infection and one of the world’s most severe TB epidemics ¹⁰. The community served by the Aurum Tembisa Clinical Research Centre (CRC) has a 30% prevalence of HIV and TB incidence of ~2500/100,000. It is therefore not surprising that a national health survey conducted in South Africa in 2004 found prior TB to be the strongest risk factor for subsequent chronic bronchitis, surpassing the risks posed by cigarette smoking and use of indoor stoves ¹¹. The authors estimated that 25% of the South African COPD burden is attributable to TB.

Recent interest has focused on the concept of using small molecule drugs to harness endogenous defense mechanisms that could, when combined with ATT, tilt the host-pathogen equilibrium in TB lesions towards resolution ¹². An ideal candidate for such host-directed therapy (HDT) would be an existing, low cost drug approved for another indication and with an excellent safety profile

and low induction of cytochrome p450 (CYP) enzymes. Two desirable, non-exclusive effects of an HDT candidate would be accelerated sterilization of TB lesions and mitigation of immune pathology resulting in less lung cavitation, less hemoptysis, less pulmonary fibrosis and less PIAT. Dr. Singhal (MPI) discovered that the antidiabetic drug metformin ameliorates lung pathology, reduces inflammation and enhances antigen-specific immunity and the efficacy of ATT in Mtb-infected C57BL/6 mice¹³. A subsequent study by Dutta et al.¹⁴ using Balb/c mice concluded that metformin does not improve the sterilizing activity of first-line antimicrobial TB treatment despite trends for lower CFU at 2 months ($P=0.54$) and 3.5 months ($P=0.039$) of adjunctive treatment. The differences in the two studies could reflect differences in mouse strain and methodologies. The study by Dutta did not include assessment of immune pathology, whereas the immunomodulatory effects of metformin might provide a more significant clinical benefit than potentially accelerated sputum conversion.

No preclinical animal model studies of metformin use in TB other than those cited above^{13;14} have been published as of October 23, 2019. We are aware of unpublished data from the Basaraba laboratory at Colorado State University indicating exacerbation of pulmonary immune pathology when metformin treatment was started in guinea pigs 4 weeks after low dose aerosol Mtb infection. This contrasted with reduced inflammation when metformin was started before infection, at the time of infection or at 2 weeks post-infection. In these guinea pig studies, metformin was given alone without concurrent antimicrobial treatment, whereas METHOD trial participants will be concurrently treated with antimicrobials.

New published and unpublished findings from the Singhal and Kornfeld labs and collaborators in the Netherlands and Colorado State University demonstrate that: (i) metformin treatment enriches nonclassical tissue repair monocytes in the blood of metformin-exposed healthy human volunteers¹⁵ and in the lungs mice (Bohme et al., manuscript in preparation); (ii) metformin rewires the metabolic and transcriptional programs of memory-like CD8+CXCR3+ T cells in mice, which promotes an enhanced response to BCG vaccination (Bohme et al.); (iii) metformin enriches memory-like CD8+CXCR3+ T cells in the blood of metformin-exposed healthy human volunteers (Bohme et al.); (i.v.) metformin decreases lung inflammation in Mtb-infected guinea pigs and enhances BCG vaccine efficacy (Bohme et al.); and (v.) adoptive transfer of metformin-educated CD8+ T cells in mice confers greater protection than sham-treated donor cells in the aerosol TB model (Bohme et al.). These results offer insights to potential mechanisms of HDT efficacy for metformin since increased nonclassical patrolling monocytes are linked to the phenomenon of trained immunity, the resolution of inflammation and tissue repair, and since CD8+ T cells are known to control acute and chronic Mtb infection, particularly in humans and non-human primates. The latter property is particularly relevant for people with CD4+ T cell deficiency due to HIV.

Retrospective data from patients with diabetes mellitus (DM) and TB presented in Dr. Singhal's report¹³ showed that use of metformin vs any other DM treatment was associated with lower risk of cavitary disease and death. Ten subsequent retrospective studies in DM cohorts variously reported that use of metformin was associated with reduced risk of latent TB infection, progression from latent infection to active TB disease, accelerated sputum conversion, reduced risk for cavitary TB, reduced mortality during TB treatment, and reduced risk for recurrent TB after treatment completion¹⁶⁻²⁵. Altogether, these studies involved 287,284 individuals treated with metformin. Several of them provided evidence that the protective effect of metformin was unrelated to glycemic control, and two of the larger studies^{18;19} assessed metformin exposure and identified a dose-dependent effect.

In addition to this retrospective evidence, there are newer reports of prospective studies that further support the potential HDT efficacy of metformin for TB. Matrix metalloproteinases (MMPs)

are implicated in lung matrix damage in TB²⁶. Data from the prospective Effects of Diabetes and Tuberculosis Severity study in India show that among patients with TB-DM comorbidity, use of metformin is associated with significantly lower levels of several MMPs linked to TB immune pathology²⁷. An unpublished observational cohort study conducted in Pune, India, evaluated 799 adults with pulmonary TB, including 225 with comorbid DM, of whom 117 (52%) were taking metformin before or after TB diagnosis. Use of metformin reduced unfavorable TB treatment outcomes by 46% (RR 0.54; 95% CI: 0.29 – 1.01, p=0.05)²⁸.

While not directly pertinent to TB, a substantial body of published studies describe protective effects of metformin against other infectious diseases in humans and animal models. Metformin was reported in several studies to improve outcomes in hepatitis C infection and reduce the risk of hepatocellular carcinoma²⁹ and to reduce the risk of bacterial sepsis³⁰ and *Clostridium difficile* infection (OR 0.58, CI 0.37-0.93; P=0.023)³¹. Compared to sulfonylurea exposure, metformin reduced the neutrophil-lymphocyte ratio (NLR) independent of glycemic control in a treatment-naïve diabetes mellitus cohort³². This may be relevant to the METHOD trial since the NLR is elevated in TB with or without DM and correlates with disease severity³³. Metformin exerts protective effects in mouse models of *Trypanosoma cruzi*³⁴ and *Trichinella spiralis*³⁵ infection and it improves survival in *Drosophila* flies with mucormycosis³⁶. The latter fly data point to host-protective effects of metformin acting on fundamental defense mechanisms. Metformin also protects against LPS-induced myocardial dysfunction in mice³⁷, which might be relevant to early mortality in TB. In various in vitro models, metformin was shown to inhibit hepatitis B virus replication in human hepatoma cells³⁸, to limit *Pseudomonas aeruginosa*-induced airway epithelial barrier damage³⁹, and to suppress *Porphyromonas gingivalis* LPS-induced inflammatory response of human gingival fibroblasts⁴⁰.

Metformin is in an outstanding HDT candidate based on the preclinical mouse and human data, its mechanism of action and its history of safety in treating DM. Metformin is eliminated unchanged in the urine and, importantly, metformin is not known to inhibit or induce any CYP enzyme⁴¹. Treatment with metformin for 3 months would add less than \$10 USD to the cost of TB treatment, which is a major consideration for Sub-Saharan Africa and other low and middle-income countries with high TB burden. Since Dr. Singhal's 2014 report in *Science Translational Medicine* there has been considerable worldwide interest in metformin as a leading HDT candidate suitable for Phase 2 clinical trials¹³. In 2019, the design for an open-label, randomized parallel arm trial of metformin added to standard ATT for pulmonary TB was published in *BMJ Open*⁴². The METRIF trial will be conducted in India and differs from the METHOD trial in several respects: the study population will be largely if not entirely HIV-negative; lung function will not be assessed; and the metformin arm will be treated with metformin IR 500 mg once daily for 1 week, followed by 1000 mg once daily for 7 weeks. No other prospective randomized clinical trials similar to the METHOD trial have been initiated or published to our knowledge.

There is a wealth of information about the use of metformin for its primary approved indication; it is the most widely prescribed drug for the treatment of type 2 DM and one of the ten most widely prescribed drugs for any indication worldwide⁴³. While there is a paucity of clinical studies of metformin safety and efficacy as HDT for TB, many clinical studies have examined the efficacy and tolerability of metformin treatment in nondiabetic, HIV-positive individuals with metabolic syndrome or with lipodystrophy associated with ART⁴⁴⁻⁵³. A meta-analysis of randomized trials using insulin-sensitizing agents to treat HIV-associated lipodystrophy included 6 trials of metformin vs placebo (N=287 participants, mean duration 27 weeks) and 3 trials of metformin vs rosiglitazone (N=152 participants, mean duration 29 weeks)³⁴. The anticipated side effect of GI intolerance with metformin occurred in both the intervention and control study arms but none of the studies included in that meta-analysis reported metformin-associated lactic acidosis (MALA).

The preponderance of evidence from 26 published studies suggests that metformin is acceptable tolerability and safety for HIV-infected individuals treated concurrently with ART.

Less is known about the tolerability and safety of metformin in combination with ATT. Interim results from the Effects of Diabetes on Tuberculosis Severity study in Chennai, India, were reported by Dr. Kornfeld (Protocol Co-Chair) and colleagues in 2016⁵⁵. All participants initiated standard ATT at the time of enrollment and 59 of 111 (53%) diabetic participants were taking metformin at baseline or started treatment with metformin by study month-2. None of the diabetic participants taking metformin had to discontinue its use or adjust dosage for any reason during the time when they were also taking directly observed ATT. These limited data support the tolerability of concurrent ATT and metformin treatment but there is a clear necessity for a Phase 2A clinical trial to determine the tolerability and safety of metformin combined with ATT plus ART as a stepping-stone to a necessarily larger Phase 3 randomized controlled trial.

Gastrointestinal toxicity is a potential barrier to the widespread use of metformin as HDT in TB/HIV co-infection. If this prevents a high proportion of METHOD trial participants from completing the planned 11-week treatment course, then metformin would not be a suitable HDT candidate regardless of efficacy. A randomized controlled trial testing extended-release (XR) vs immediate-release (IR) formulations for type 2 DM treatment reported mild GI toxicity (nausea, vomiting, diarrhea) in 18.9% of participants in taking 1500 mg of metformin XR daily⁵⁶. Symptoms of nausea, vomiting and diarrhea typically peak at the initiation of metformin and improve with continued treatment. In the aforementioned trial, 5 of 532 participants (0.6%) in the combined metformin XR arms (1500 mg daily, 750 mg BID, 2000 mg daily) discontinued the drug due to GI events in the first week of treatment vs 7 of 174 (4.0%) in the metformin IR arm. However, owing to withdrawal of XR formulations from the market, the METHOD trial uses the IR product metformin hydrochloride made by Mylan Pharmaceuticals, Inc., starting at a dose of 500 mg daily and increasing to a maximum of 500 mg twice daily that we expect will be tolerated.

Participants in the METHOD trial will receive ATT and ART following the standards of the Republic of South Africa Department of Health and the Southern African HIV Clinicians Society⁵⁷. Drug-resistant TB is an exclusion criterion, so all participants will receive the standard 6-months course of first-line ATT (2([HRZE])/4[RH]). Participants on ART at enrollment will continue that throughout the trial. Participants who are ART-naïve at enrollment will begin ART within 2-8 weeks of ATT initiation, at the discretion of public health clinic medical staff. This conforms to the following the current standard Universal Test and Treat protocol adopted South Africa as applied to TB co-infected patients. Baseline CD4+ T cell count <50 cells/mm³ is an exclusion, so ART does not need to be started coincident with ATT initiation. First-line ART in South Africa previously comprised the nucleotide analog reverse transcriptase inhibitor tenofovir (TDF) plus the nucleoside reverse transcriptase inhibitor emtricitabine (FTC) plus the non-nucleoside reverse transcriptase inhibitor efavirenz (EFV). Dolutegravir (DTG; integrase strand transfer inhibitor) has replaced EFV in the recommended first-line regimen for South Africa, but EFV is preferred for women seeking to conceive and for continuation in those already taking it prior to initiating ATT.

The conventional goal for HDT candidate drugs is to achieve durable cure of active TB disease (low relapse rates) with ATT regimens shorter than 6 months. Definitive evidence of efficacy for treatment shortening will require Phase 3 trials with very large cohorts and at least 12 months of follow-up after ATT completion to capture TB recurrence. A trial of that scope is unfeasibly costly to propose without foundational evidence for the tolerability and safety of metformin in the target population, as well as a signal indicating likely efficacy. The METHOD trial will test whether metformin is safe for use in people treated concurrently for TB and HIV, and whether it exhibits potential for TB treatment shortening or a beneficial reduction of PIAT.

The chief microbiological outcome for the METHOD trial is time to stable sputum culture conversion in liquid media. This is a feasible and proven method to efficiently demonstrate a biologically and clinically relevant impact of metformin with promise for treatment shortening. Its use is supported by results of the PanACEA MAMS-TB trial that tested four new potential ATT regimens at seven sites in South Africa, including Tembisa⁵⁸. That trial detected a significantly shorter time to liquid culture conversion (48 vs 62 median days of conversion or HR=1.75; P=0.01) in the high-dose rifampicin arm compared to standard therapy. There was a corresponding reduction in the proportion positive solid medium cultures at month-2 but the difference was not statistically significant. Sputum solid culture status at month-2 will be included in the METHOD trial as a complementary approach to evaluate the potential for treatment shortening. The sample size for PanACEA MAMS-TB was 57-63 participants per arm in different treatment arms and 123 in the control arm. The size of the METHOD cohort (n=50 per arm with evaluable data) is selected to address the primary goal of testing tolerability and safety. While smaller than PanACEA MAMS-TB, the METHOD cohort may be sufficient to identify a positive trend for efficacy based on the anticipated effect size of metformin.

Most morbidity and mortality in TB results from collateral lung damage caused by the host immune response, including inflammation and fibrotic resolution. Immunomodulation concurrent with antimicrobial treatment for infection can be safe and host-protective as demonstrated by the use of corticosteroids to improve *Pneumocystis jirovecii* pneumonia outcomes. Limiting lung damage in TB could be an HDT effect of equal or even greater importance than accelerated sputum sterilization. Accordingly, the change in FEV1% from baseline to TB treatment completion is a secondary outcome in the METHOD trial. Spirometry values in TB patients are typically lowest at the time of diagnosis and improve, to variable extent, with treatment⁵⁹. Lung function and exercise capacity will be further quantified using well-validated and widely used parameters of spirometry (FVC, FEV1/FVC)⁶⁰ and the 6-minute walk test⁶¹ with pulse oximetry⁶². Respiratory quality of life (QoL) will be quantified using the St. George's Respiratory Questionnaire (SGRQ). This health-related QoL instrument has been validated for a variety of lung diseases including pulmonary TB⁶³ and it is currently being used in an observational TB study in South Africa⁶⁴.

Enrollment in the METHOD trial will not be limited by stratification, but strata will be analyzed post hoc. Participants will be classified at enrollment by radiographic extent of disease using the simple 3-level scale developed by the NTRDA⁶⁵. Natural history studies in the pre-chemotherapy era using this scale found that the risk of death due to TB in the 10 years post-diagnosis increased progressively from 12% to 21% and 68% as radiographic extent of disease at TB diagnosis increased from minimal to moderately advanced and far advanced⁶⁶. A study of 71 South African TB patients conducted in 1989 found the extent of lost respiratory function, measured up to 16 years after curative ATT was directly correlated with the radiographic extent of disease at diagnosis⁶⁷.

The categorical NTRDA x-ray scale used for enrollment classification in the METHOD trial will not be used to assess the effects of treatment, a task for which the number of levels is insufficient. To assess treatment effects, we will use a more recently described, validated numerical score to grade chest x-ray severity developed by Ralph et al.⁶⁸. This linear variable will be used to quantify the radiographic extent of pulmonary TB disease at enrollment and the degree of resolution at study completion. Two blinded, trained x-ray readers at the University of Massachusetts Medical School (UMMS) will score the digital chest x-ray images by the categorical and numerical approaches. Disagreement between the two independent scores will be reconciled by the two readers plus a third, trained adjudicator before the final score is entered in the Radiology case report form (CRF). The METHOD trial will employ a training process developed by Dr. Kornfeld,

in collaboration with Indian collaborators for the RePORT India Consortium. Numerical x-ray severity score data from readers trained in this way was used successfully to identify differences in the degree of resolution between x-rays performed at baseline and after ATT completion in TB patients with and without diabetes ⁵⁵.

6.2 Rationale

Preclinical data in mice show that metformin (1) enhances macrophage killing of Mtb by activating AMP-activated protein kinase (AMPK) and downstream effector pathways, (2) enhances CD8⁺ T cell responses, and (3) reduces inflammation associated with TB disease ¹³. Because these mechanisms contribute to immune protection and the control of bacterial replication, it is expected that metformin will enable treatment shortening for TB. Data from a number of retrospective studies show that use of metformin is associated with reduced risk to develop latent TB infection, reduced risk to progress from latent infection to active TB disease and reduced risk of mortality during TB treatment ^{13,16-25}.

These data were obtained in diabetic populations, but metformin demonstrated efficacy after adjustment for glycemic control and when compared to other anti-diabetic drugs. Recent studies describing accelerated sputum conversion with metformin have particular relevance to the METHOD trial. Santos et al. ²⁰ reported that diabetic TB patients taking metformin vs non-metformin regimens had significantly reduced time to sputum conversion (50 vs 73 days) despite no difference in glycemic control. Lee et al. ²¹ reported accelerated sputum conversion with metformin but only in cases of cavitary TB. Ma et al. ²² reported increased month-2 culture conversion with metformin. Based on published mouse and human data demonstrating the efficacy of adjunctive metformin through defined mechanisms of action, and its outstanding safety record in widespread clinical use for approved indications, there is a compelling rationale to test metformin in combination with ATT to accelerate sputum culture conversion and to reduce damaging immune pathology during TB treatment. Shorter TB treatment regimens are expected to improve adherence, reduce cost, and reduce the risk of acquired drug-resistance ⁶⁹. Modulation of immune pathology is expected to reduce the toll of PIAT in the predominantly working-age population of TB patients. These considerations support the objectives of the METHOD trial.

Metformin is approved worldwide for the treatment of DM; its use as HDT for TB is investigational. Treating non-diabetic people with metformin is also investigational but, unlike the case for HDT activity, this has been extensively tested for other non-FDA-approved conditions including pre-diabetes, HIV-related lipodystrophy, infertility, polycystic ovary syndrome and gynecological cancers ^{52,70,71}. Metformin reduces hepatic glucose production and increases insulin sensitivity but unlike other oral anti-diabetic agents, metformin has very low potential to provoke hypoglycemia in non-diabetic individuals. Finally, there is abundant evidence for acceptable tolerability and safety of metformin in people who are HIV-infected and taking ART. Metformin carries a “black box” warning for lactic acidosis (LA) as a potential life-threatening adverse effect but the legitimacy of this association is controversial ⁷². Risks are minimized in the METHOD trial cohort by excluding people with known risk factors for LA (Section 9.1.2). No other candidate agents in the HDT pipeline match the pre-clinical and retrospective clinical evidence of efficacy established for metformin and very few match its low cost. These considerations support the feasibility of the METHOD trial.

There is currently no solid basis in evidence to determine the optimal dose or duration of metformin treatment for HDT efficacy against TB, so these parameters for the METHOD trial were selected based on the best available indirect evidence. A metformin dose 250-500 mg/kg in

drinking water was required to see protective effects against TB in C57BL/6 mice¹³. Humans treated for DM with typical metformin doses of 1.5-2.5 gm daily have plasma levels in the 10 µM range⁷³. Metformin administered to C57BL/6 mice at 250 mg/kg in drinking water for 2 weeks resulted in plasma concentrations in the 5 µM range⁷⁴, supporting the relevance of the mouse data to human TB treatment. The relative efficacy of metformin to reduce rates of progression from TB infection to disease and mortality of TB disease in patients with type 2 DM was shown to be dose-dependent in two retrospective studies^{18;19}, suggesting that a dose below 1000 mg daily might not show HDT efficacy. However, a study by Lachmandas et al.¹⁵ reported changes in systemic immune parameters of healthy Dutch adults treated with metformin for six days in escalating doses that peaked at 2000 mg for a single day and ended with 1000 mg on the last day. The mean daily dose in that study was 1167 mg. In view of that data, and since African Americans are more sensitive than European Americans to the anti-diabetic effects of metformin⁷⁵, a dose that is equivalent to range between 1000 mg to 1500 mg appears to offer the best chance of showing efficacy while minimizing risk in the METHOD cohort.

Dolutegravir inhibits OCT2 and was reported to increase metformin exposure substantially (79% higher AUC)⁷⁶. Concurrent use of metformin and DTG was reported to have acceptable tolerability and safety^{77;78}.

All METHOD trial participants will take rifampicin as part of the standard 2[HRZE]/4[RH] regimen for drug-sensitive pulmonary TB. Rifampicin increases metformin exposure by ~28%, due to increased intestinal absorption⁸⁰.

Trimethoprim inhibits OCT2 and MATE1 to a moderate degree (~37% increase in AUC at a dose of 200 mg every 12 hours)⁸⁰. The prophylactic dose of trimethoprim in HIV is 160 mg once daily, which is estimated to increase metformin exposure by ~15%. Since Pneumocystis prophylaxis is recommended for CD4+ T cell count <200 cells/mm³ it is expected that some METHOD trial participants will be taking trimethoprim, which could increase metformin exposure by up to ~122% if the combined effects of trimethoprim, DTG, and rifampicin are additive. Participants reporting the use of any drug on the list of precautionary medications other than trimethoprim will be advised to consider alternative agents in consultation with their medical providers.

Based on the interaction with rifampicin, the daily dose of metformin used in the METHOD trial (1000 mg) is expected to result in plasma drug levels equivalent to an oral dose of 2070 mg or 1280 mg for DTG or EFV regimens, respectively, and 2220 mg if trimethoprim is also taken concurrently. These levels are below the maximum recommended daily dose for diabetes treatment (2550 mg) but above the mean dose in the Lachmandas study, which reported changes in blood mononuclear cell metabolism, gene expression and cytokine production¹⁵. Metformin at the mean dose used by Lachmandas (1167 mg) also increased mitochondrial mass, increased non-classical monocytes associated with trained immunity, and increased the capacity of mononuclear phagocytes to restrict Mtb replication ex vivo¹⁵ suggesting the potential for HDT efficacy. If any METHOD trial participant is also taking trimethoprim prophylaxis, that could result in plasma metformin levels up to 2440 mg. Such an increase remains within safe bounds and should not confound the assessment of safety and efficacy endpoints.

Participants in the METHOD trial will take the study drug for 11 weeks, which provides metformin exposure through the intensive phase of ATT plus one additional month. This time period corresponds to the peak of bacterial burden and inflammation when metformin is expected to have its greatest beneficial impact. The study testing in vivo effects of metformin on immunity to Mtb in healthy human volunteers identified potentially protective biological responses within the first 3-6 days of treatment¹⁵. On this basis, the risk of prolonged metformin exposure with

extended dosing through the entire 6-month course of ATT is unjustified when weighed against potential benefit. For the planned METRIF trial in India, participants in the intervention arm will be treated with metformin 1000 mg daily for 2 months⁴². A trial of multiple HDT candidate (vitamin D2, everolimus, auranofin and CC-11050) sponsored by the Bill and Melinda Gates Foundation and conducted at Tembisa used a treatment period of 4 months. Clearly, no standard for the optimal duration of any TB HDT regimen currently exists; the 3-month duration used in the METHOD trial provides a reasonable balance between the potentially conflicting goals of ensuring participant safety and demonstrating HDT efficacy.

Comparison of the metformin arm and standard treatment arm of the METHOD trial includes an investigation of metformin effects on selected plasma and serum proteins, the whole blood transcriptome, and on cell surface phenotypes of leukocyte subsets that may reflect the biological activity of metformin as HDT for TB. Results of this study may be mechanistically informative with respect to protective efficacy (or lack thereof) when analyzed in the context of the secondary endpoints of the interventional study (sputum conversion and respiratory health measures) as well as whole blood assay and PK/PD results.

Results of this systems immunology study could also identify biomarkers that could improve the productivity of a multi-site phase 3 trial of metformin for TB HDT where different sites might lack resources (e.g. spirometry) used in the METHOD trial. Inclusion of the HIV⁺/TB⁻ omics control group is necessary to disambiguate the influence of HIV vs TB on the parameters that will be analyzed.

These control participants will provide the baseline information on any possible perturbation which HIV might have on immune cell gene expression, which could significantly affect the immune parameters among HIV⁺/TB⁺ patients in the two study arms. The data from HIV⁺/TB⁻ omics control group will be used to normalize the impact of HIV infection in the treatment arms.

6.3 Study Hypotheses

Hypothesis #1: Metformin has acceptable tolerability and safety when combined with ATT and ART in patients co-infected with TB and HIV.

Hypothesis #2: Metformin combined with standard ATT accelerates sputum conversion and improves respiratory outcomes of pulmonary TB disease in HIV co-infected individuals.

The omics control group for the METHOD trial supports investigations that are linked to the second hypothesis.

7 OBJECTIVES

7.1 Primary Objectives

The primary objectives of the METHOD trial are:

1. Determine tolerability and safety of metformin added to standard ATT and ART in the treatment of TB/HIV co-infection.
2. Conduct a pharmacokinetic-pharmacodynamic analysis of metformin toxicity, efficacy, and immune modulation.

The objective of the Omics Control Group is to provide baseline values for systems immunology investigations performed with samples from the treatment arms of the METHOD trial.

7.2 Secondary Objective

To evaluate the potential efficacy of metformin as adjunctive therapy when added to standard ATT and ART for TB/HIV co-infection. Efficacy will be determined based on the parameters listed as secondary endpoints.

7.3 Exploratory Objectives

The exploratory objective of the METHOD trial is to evaluate the potential of metformin to exert host-protective effects against HIV based on the following measures:

1. Change in viral load from baseline to study drug completion.
2. Change in CD4+ T cell count from baseline to study drug completion.
3. Occurrence of TB-associated immune reconstitution inflammatory syndrome (TB-IRIS).

8 STUDY DESIGN

The METHOD trial will be a Phase 2A randomized, open-label trial of metformin added to standard ATT and ART in HIV-positive participants with drug-sensitive pulmonary TB disease. Two sites, the Aurum Tembisa CRC and Isango Lethemba TB Research Unit operated by The Aurum Institute and Wits Health Consortium, will be used for the METHOD trial unless accrual does not meet expectations, in which case other sites in South Africa operated by The Aurum Institute will be added. There are two parallel arms for this interventional trial: standard treatment for TB/HIV coinfection vs metformin added to standard treatment. Enrollment in the METHOD trial will not be limited by stratification, but strata will be analyzed post hoc. Participants will be classified at enrollment by radiographic extent of disease using the simple 3-level scale developed by the NTRDA. Block randomization will be performed on participants conforming to the inclusion and exclusion criteria listed in Sections 9.1.1 and 9.1.2.

The cohort will comprise 112 participants with TB/HIV co-infection (56 in each study arm). The site will begin enrolling participants in the fourth quarter of funding year-2 and enrollment will be completed in the fourth quarter of funding year-4, allowing 27 months for enrollment. The accrual goal will be met if 4 participants are enrolled per month, which is well within enrollment rates achieved for previous studies conducted at the Aurum Tembisa CRC. Individual participation spans 9 months, including 6 months of clinic follow-up visits and final telephone interview at week-36. With the final visit of the last-recruited participant occurring in the third quarter of year-5. We plan total enrollment of 112 HIV+/TB+ individuals to make up for instances of early withdrawal and default with a goal of retaining 100 participants with evaluable data for analysis. To provide baseline values for proteomic, transcriptomic and flow cytometry studies, we will recruit HIV+ controls without active TB disease (N=30) with participation limited to a single time point. These omics control participants will come from the same general population as the HIV+/TB+ participants in the METHOD cohort. These omics control participants will only be enrolled at the Tembisa CRC. The HIV status of the omics control group will be confirmed by POC HIV serology. Absence of pulmonary TB disease in omics participants will be screened by sputum Xpert and, if negative, confirmed by negative chest x-ray and sputum Mtb culture. The maximum total enrollment including both study arms and omics control group is 142 individuals.

The primary objective of the METHOD trial is to determine whether metformin can be tolerated when used concurrently with standard ATT and ART. The primary endpoint is the number of grade 3 or 4 adverse events during the time on study drug plus 4 weeks post study drug completion. Based on clinical experience with metformin used to treat DM, the most common cause of adverse events will be GI toxicity. Manifestations of GI toxicity include nausea, vomiting and diarrhea. Follow-up visits occur weekly for the first two months of study participation when the risk of drug intolerance is highest. Study drug may be prematurely discontinued at any time based on criteria listed in Section 13.6.1.

The secondary endpoint reflecting microbiological efficacy is time to conversion of sputum Mtb culture using the Mycobacterial Growth Indicator Tube (MGIT) system. We will also test the complementary endpoint of month-2 sputum solid culture status. The selection of time to conversion follows the approach implemented in the PanACEA MAMS-TB trial⁵⁸ that had treatment arms of comparable size to the METHOD trial. Baseline and month-2 sputum samples collected for culture on solid media are included based on data linking month-2 sputum status to treatment outcome (see http://cid.oxfordjournals.org/content/61/suppl_3/S160.long). Participants having negative solid cultures at baseline or with contaminated cultures at month-2 will be excluded from the month-2 analysis but will still be included in analysis of MGIT results if baseline

MGIT was positive. Some participants are expected to show discordant results of solid culture and MGIT during the first two months of treatment, with MGIT showing greater sensitivity⁸¹ but higher rates of contamination.

Efficacy will be evaluated based on a modified intention-to-treat population, consisting of all participants who underwent randomization and received the study medication at least once, but excluding participants whose initial laboratory evaluation subsequently indicated study ineligibility due to negative baseline Mtb culture or positive baseline culture with resistance to isoniazid, rifampicin, or pyrazinamide. The modified intention-to-treat analysis will include all participants who have taken at least one dose of the study medication during the continuation phase of ATT. This group will be treated as the any-exposure population. In addition, participants who took at least 80% of all doses over a period of 3 months will be included in a per-protocol population. A secondary analysis will examine efficacy endpoints in this population. In view of the potential for discordant results between solid and MGIT cultures, participants with negative solid cultures at baseline or with contaminated solid cultures at week 8 will be excluded from the month-2 analysis but will still be included in analysis of MGIT results if baseline MGIT was positive, and they will be included in the secondary outcomes analysis.

Omics control group participants will be evaluated at the Aurum Tembisa CRC, with a screening visit to establish eligibility followed by an enrollment visit within 14 days when samples will be collected for additional clinical and research laboratory testing. Participants in the omics control group will be recruited from the same general population as the HIV⁺/TB⁺ participants in the METHOD trial treatment arms. Thus, their HIV status may be known prior to screening and in some instances a recent sputum Xpert TB/RIF result and/or chest x-ray may be available. HIV-seropositivity will be confirmed by point of care (POC) HIV testing if not on ART. Sputum Xpert will be performed on omics control group participants at screening and, if negative, the absence of active pulmonary TB will be confirmed by chest x-ray and sputum Mtb culture.

A total of 30 individuals will be enrolled in the omics control participant group. This sample size matches the N=30 participants from each of the two treatment arms whose samples will be used for systems immunology evaluations.

9 STUDY POPULATION

The METHOD trial study population will comprise of adult (18-65 years) female and male participants residing in the greater catchment area of the Aurum Tembisa CRC, situated in City of Ekurhuleni District, the City of Johannesburg, City of Tshwane District, within the Province of Gauteng, and Nelson Mandela Bay area, Sub district A and C within the Province of Eastern Cape, South Africa. Based on experience with other clinical trials conducted at the Aurum Tembisa CRC, we anticipate that the cohort will comprise 50% females with median age 37 years and the following proportion of ethnicities: 97% Black/African, 1% Coloured, 1% Indian/Asian, 1% White/European. The population at the Port Elizabeth site has different characteristics, so the final composition of the METHOD cohort will be influenced by the proportion of participants enrolled from Gauteng versus Port Elizabeth.

9.1 Inclusion/Exclusion Criteria

9.1.1 Participant Inclusion Criteria

Participants must meet all of the following inclusion criteria to enroll in the treatment arms of the METHOD trial:

1. Age 18 through 65 years.
2. HIV-1 seropositive status prior to or after screening.
3. Chest radiograph compatible with pulmonary TB.
4. Positive sputum Xpert TB/RIF or ULTRA with one Ct <25 OR subsequent culture confirmation⁸².
5. RIF susceptibility diagnosed by an approved TB diagnostic test e.g. Xpert TB/RIF, ULTRA or BD Max
6. Residence within study catchment area.
7. If female of childbearing potential, willing to use contraception for the duration of study participation (criteria for childbearing potential and for acceptable contraception are listed in Section 11.3.6). If male, willing to use condoms for the duration of metformin treatment plus an additional three months after stopping metformin.
8. Able and willing to provide informed consent (IC).

Inclusion criteria 1, 2, 6, 7, and 8 also apply to participants in the omics control group.

9.1.2 Participant Exclusion Criteria

Candidates for participation in the METHOD trial will be excluded if they meet any of the following exclusion criteria:

1. Any condition for which participation in the trial, as judged by the investigator, could compromise the well-being of the participant, or prevent, limit, or confound protocol-specified assessments.
2. Is critically ill, and in the judgment of the investigator has a diagnosis likely to result in death during the trial or the follow-up period.
3. TB meningitis or other forms of severe TB with high risk of a poor outcome as judged by the investigator.
4. Pregnant or breastfeeding.
5. Resistance to any first-line ATT drug demonstrated by susceptibility testing.
6. More than 14 days ATT for the current episode of TB, prior to enrollment.

7. Taking any fluoroquinolone antibiotic.
8. History of diabetes mellitus or fasting blood glucose ≥ 7.0 mmol/L on screening evaluation.
9. History of congestive heart failure, chronic liver disease, autoimmune disease, or malignancy.
10. Consumption of >28 units (men) OR >21 units (women) of alcohol/week
11. Use of metformin within 1 year prior to enrollment.
12. History of sensitivity to metformin.
13. Acute or chronic metabolic acidosis based on reported medical history or laboratory tests performed on screening evaluation.
14. Body mass index (BMI) <17.0 kg/m² on screening evaluation.
15. Peripheral blood CD4+ T cell count <50 cells/mm³ on screening evaluation.
16. Hemoglobin <9 g/dL (men) or 8 g/dL (women) on screening evaluation.
17. Platelet count $<50,000$ /mm³ on screening evaluation.
18. Absolute neutrophil count <750 cells/mm³ on screening evaluation.
19. Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² calculated by the CKD-EPI equation.
20. Serum bicarbonate <18 mmol/L on screening evaluation.
21. AST or ALT ≥ 3 times the upper limit of normal (ULN) on screening evaluation.
22. Hepatitis B surface antigen positive.
23. Enrollment in another interventional study at any time during participation in the METHOD trial.
24. Imprisonment at the time of or after enrollment in the METHOD trial.
25. Diagnosis of active COVID-19 at time of screening or high suspicion of active COVID-19 disease during screening.

Exclusion criteria 1, 2, 4, 7, 8-11, and 13-25 also apply to participants in the omics control group. A positive sputum Xpert TB/RIF or Mtb culture, or radiographic evidence of any pulmonary infectious disease process are exclusion criteria for the omics control group.

9.1.3 Co-enrollment Criteria

Participants in the METHOD trial are prohibited from co-enrollment in any other interventional human clinical research study at any time from screening through the final study visit. Co-enrollment in an observational study is permitted only with the approval of the national PI or protocol Chair of the METHOD study and the observational study.

9.2 Recruitment Process

Tried and tested methods will be used to identify TB suspects. Aurum's Tembisa CRC and Isango Lethemba TB Research Unit has a long standing and existing relationship with the NHLS, the national reference laboratory of South Africa providing diagnostic and testing services to $>80\%$ of the South African population. This relationship has been strengthened through the Advancing Care and Treatment for TB and HIV Collaborating Centre (ACT4TB/HIV) led by Aurum, as well as the Wits Health Consortium.

9.2.1 Potential candidates for participation in the treatment arms:

Candidates eligible for participation in the trial will be identified as TB positive and HIV positive. They will be recruited from Primary Health Care clinics in four districts: **1**, Ekurhuleni (27 clinics);

2, City of Johannesburg, Regions A (8 clinics) and Region E (4 clinics) and; **3**, Tshwane, Region 3 (12 clinics), Region 4 (5 clinics), Region 5 (2 clinics), and Region 6 (8 clinics), Nelson Mandela Bay area, Sub-district A (18 clinics) and Sub-district C (12 clinics). Candidates will be identified using 5h3 TB Suspect Registry. With consent from the potential participant, we will trace Xpert MTB/RIF results using the National Health Laboratory Service (NHLS) TrakCare web portal. This recruiting plan was approved by the South African Health Products Regulatory Authority (SAHPRA) and the University of Witwatersrand Human Research Ethics Committee (WHREC).

HIV status and CD4+ T cell count will be known for some but not all participants prior to screening. The HIV results will be accessed using Tier.Net for candidates with previously known HIV status. The primary care clinic staff for Aurum and Isango Lethemba TB Research Unit staff (with permission from the District Department of Health) will introduce/refer selected candidates (with their permission) to the Aurum Tembisa CRC study team and Isango Lethemba TB Research Unit, allowing the team to look up additional laboratory results and initiate recruitment. Recruiting will also be implemented through the HIV Counselling and Testing program operated by Aurum in the Tembisa catchment areas.

9.2.2 Potential candidates for participation in the omics control group:

Participants will be identified as TB negative and HIV positive. Recruitment strategy will utilize the same clinics as above. All candidates will have negative Xpert results from the National Health Laboratory Service available before screening, and many will have positive HIV serology and viral load results. Where HIV test results are unavailable, this will be performed at screening. Sputum mycobacterial culture and chest x-ray will be done to confirm that absence of active TB disease. For all control participants, the site will assess the requirement for referral for IPT.

9.3 Participant Retention

The staff at the Aurum Tembisa CRC includes a Community Engagement Unit comprising a Community Engagement Manager, a Recruitment & Retention Leader, a Retention Officer and two Recruiters and the Isango Lethemba TB Research Unit team comprises of field workers/counsellors and study coordinators. Through the effort of these individuals and other members of Aurum Tembisa CRC team, retention in nine completed or ongoing trials between 2008 and the present has ranged from 85 to 100%. To minimize loss of follow-up, participants receive a phone call reminder one week before scheduled visits and the call is followed by an SMS reminder. A phone call and SMS reminder are made again one day prior to the scheduled visit. If an appointment is missed, the participant is visited at home and transportation arranged, if necessary. Retention is also supported by making the Aurum Tembisa CRC and Isango Lethemba TB Research Unit waiting areas and exam areas inviting, clean and professional with television and refreshments available. Participants are reimbursed R300 per visit, per SAHPRA and WHREC recommendations. Participants in the sparse PK sampling group will be reimbursed R450 and those in the intensive PK sampling group will be reimbursed R1000 at V06 (week 5).

10 INTERVENTIONS

10.1 Biomedical Interventions

Treatment with metformin is the only intervention used in the METHOD trial. All participants will receive standard ATT, ART and prophylaxis for opportunistic infection according to local and national standards.

10.1.1 Regimen (dose, schedule, route, administration)

Metformin hydrochloride 500 mg tablets once daily by mouth for 1 week, escalating to 500 mg twice daily for 10 weeks in the metformin arm only. It should be taken with a meal and the tablet should be swallowed whole and never crushed or chewed.

10.1.2 Study Product Formulation and Preparation

Metformin hydrochloride 500 mg tablets will be used. These tablets contain the inactive ingredients artificial blackberry flavor, magnesium stearate, polyethylene glycol, polyvinyl alcohol, povidone, talc, and titanium dioxide. Recommended storage temperature is 20-25 °C.

10.1.3 Study Product Supply and Accountability

Metformin hydrochloride 500 mg (NDC 0378-7185-05) manufactured by Mylan Pharmaceuticals, Inc.

The recommended storage temperature range is 20-25 °C.

The receipt, handling, storage, dispensing accountability, and destruction (where applicable) of study product is the responsibility of the Site Pharmacist. Refer to the Pharmacy Manual (Appendix C) for a detailed description of the study product supply and accountability.

10.1.4 Assessment of Participant Adherence with Study Product

Prior to starting metformin, participants will be counselled about the importance of following the instructions for its use, including the time of day when tablets should be taken and to take them with food. This contrasts with the recommendation for taking ATT on an empty stomach for optimal absorption of rifampicin. Participants will be informed about symptoms of potential side effects and the circumstances under which metformin should be discontinued. Self-reported adherence is checked and reinforced at every scheduled participant contact, and counselling about adherence is repeated at each visit. On an as-needed basis, staff will provide additional assistance such as intensive counselling, food parcels and phone alarms to assist participants having issues with adherence.

Metformin is provided in 30-tablet blister packs and dispensed in numbers that exceed the number of tablets that should be taken before the next visit. At follow up visits, the participant will return all used and unused blister packs to pharmacy. Pill count is done, and returns are entered on an accountability log. The number of remaining tablets is counted at each visit. If the pill count does not match the prescribed use, participants will be questioned regarding the reason and receive intensified counselling. If a participant does not return their metformin containers, this will be

followed up by the clinic and feedback tracked by pharmacy. These adherence measures are significantly more robust than those offered by local public health clinics.

10.1.5 Concomitant Medications and Procedures

Use of concomitant medications will be reviewed and recorded at baseline and at each follow-up visit through week-16 (V12), coinciding with the final adverse event (AE) assessment 4 weeks after study drug completion. METHOD trial personnel will provide TB and HIV treatment for participants while they are in the study, but participants will go to their respective clinics for extra CD4 and viral load tests as required by local and national guidelines.

Use of metformin will be temporarily suspended for any surgical procedure (except minor procedures that do not require restricted intake of food and fluids) and will not be restarted until the participant's oral intake has resumed and renal function has been evaluated as normal. A similar approach will be taken if participants coincidentally develop shock or other manifestations of critical illness from any cause.

10.1.6 Permitted Medications and Procedures

METHOD trial participants are permitted to use first-line medications for the treatment of HIV and the treatment or prevention of concurrent acute or chronic comorbidities (e.g. prophylaxis for HIV-related opportunistic infections such as trimethoprim-sulfamethoxazole, oral antifungal agents for mucosal candidiasis) and to use oral or parenteral contraceptives as stated in Section 11.3.6. If the participants are advised by HIV clinic staff to change their ART regimen, their eligibility for continued participation in the METHOD trial will be reviewed.

10.1.7 Prohibited Medications and Procedures

Use of fluoroquinolones is prohibited since drugs in this class can precipitate hypoglycemia, which could be further exacerbated when given concurrently with metformin.

10.1.8 Precautionary Medications and Procedures

Metformin is a highly polar drug; its elimination by renal tubular secretion depends on membrane-bound cation transporters (OCTs) and multidrug and toxin extrusion transporter (MATEs) whose activity may be inhibited by a number of commonly used drugs. Caution is advised for concomitant use of furosemide, nifedipine or cationic drugs eliminated by renal tubular secretion (amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that may alter metformin exposure. Rifampicin also increases metformin exposure by a mechanism that has been conclusively identified but is suspected to be increased GI absorption⁷⁹. Of these drugs, all METHOD trial participants will be taking rifampicin and, while not expected, concurrent use of trimethoprim would be permitted. The rationale for study drug selection based on expected drug-drug interactions is described in Section 6.2.

Use of metformin will be temporarily suspended if radiological studies involving intravenous iodinated contrast materials are advised for any METHOD trial participant. Metformin will be temporarily discontinued prior to the procedure and restarted no sooner than 48 hours after the procedure and only when renal function has been re-evaluated and found to be normal. No interaction is expected between metformin and EFV.

10.1.9 Required Medications

Required medications include the standard first-line ATT drugs as well as any ART drugs and prophylaxis deemed necessary by the public health clinic providers responsible for managing HIV treatment in METHOD trial participants. Treatment of TB will be managed by the METHOD trial team while treatment of HIV will be managed by government clinic providers. All METHOD participants will have drug-sensitive pulmonary TB and receive standard (2([HRZE]/4[RH]) using weight-based doses of RIFAOUR e-275 [150(75/700/275) mg] and RIFINAH [(150/75) mg] tablets. Following the local standard of care, adherence to ATT will be monitored by pill count and not by directly observed therapy. Follow-up visits occur weekly through the intensive phase of TB treatment, allowing for close monitoring and counselling to maximize adherence. First ART regimens used by providers in the Aurum Tembisa and Isango Lethemba TB Research Unit catchment areas are the combination of TDF, FTC, and DTG or EFV. Participants in the treatment arms will receive vitamin B6 25 mg to reduce the risk of deficiencies associated with isoniazid⁸³. Metformin hinders absorption of dietary vitamin B12, but clinically significant reduction in B12 levels is not expected to occur with the short duration of metformin treatment in the METHOD trial⁸⁴.

Metformin GI toxicity is minimized by taking the drug with food, so participants will be advised to take the metformin with breakfast. Participants will be advised to take ATT drugs on an empty stomach, since this facilitates rifampicin absorption. Therefore, ATT and study drugs will be taken at different times of day. Adherence to treatment and the timing of medication ingestion will be reviewed at every follow-up encounter.

10.1.10 Rescue Medications

The occurrence of MALA is a potentially life-threatening emergency. There is no medication that directly prevents MALA and no specific rescue agent. Treatment consists of supportive care including discontinuation of metformin, bicarbonate infusion and optimizing ventilation to compensate for metabolic acidosis. In severe cases, hemodialysis can accelerate the correction blood pH and osmolarity while also removing metformin and lactate from the circulation⁸⁵. METHOD trial participants with signs and symptoms of a serious adverse event (SAE) requiring urgent or emergent inpatient medical care will be transported to Lenmed Zamokuhle Private Hospital or the Tembisa Tertiary Public Hospital, which is located less than 1 km from the Aurum Tembisa CRC at 128 Flint Mazibuko Street, Hospital View, Tembisa and Livingstone Hospital or Netcare Greenacres hospital which are both located 30km from the site. These hospitals offer hemodialysis services under the direction of a resident nephrologist.

Asymptomatic and mildly symptomatic hypoglycemia (blood glucose <3.9 mmol/L) will be treated with glucose 20 gm PO. Severely symptomatic hypoglycemia will be treated with glucagon 1 mg SC.

10.2 Behavioral Intervention(s)

Behavioral interventions employed in the METHOD trial will include network-orientated peer education for adherence with ATT and ART, prevention of TB and HIV transmission, and adherence with the study drug. This support will be provided by study nurses and Community Engagement staff at every participant encounter and, if necessary, by mobile phone contact or staff visits to the participant's dwelling.

11 STUDY PROCEDURES/EVALUATIONS

11.1 Clinical Evaluations and Procedures

11.1.1 Medical history:

Medical history will be obtained by interview at screening and enrollment, and it will be updated at all follow-up visits. This information will be recorded in CRFs.

Medical history at **screening** will cover the demographic, behavioral and medical history elements listed in the Inclusion Criteria (Section 9.1.1) and Exclusion Criteria (Section 9.1.2).

Medical history at **enrollment** will cover additional details of HIV infection as reported by the participant (year of diagnosis, occurrence of AIDS-defining illness) and history of any prior episodes of TB disease (year of diagnosis, site[s] of infection, treatment). Medical history on **follow-up** visits will address the interval occurrence of elements listed in the Inclusion Criteria (Section 9.1.1) and Exclusion Criteria (Section 9.1.2) as well any outpatient or inpatient encounters with medical providers other than METHOD trial staff. A complete review of systems will be performed at the enrollment and all follow-up visits, covering all 14 systems recognized by the Centers for Medicare and Medicaid Services.

11.1.2 Medication history:

Medication history at **screening** will cover the drugs listed in the Exclusion Criteria (Section 9.1.2). Medication history at **enrollment** will cover prior or current use of ART drugs (drug names, when prescribed, self-reported compliance, adverse effects, and tolerability) use of ATT drugs for <14 days for the current episode of TB or duration for any prior episode of TB (drug names, when prescribed, self-reported compliance, adverse effects and tolerability), and all other prescription and non-prescription drugs used by the participant. Participants will be asked about the use of precautionary medications (Section 10.1.8). Medication history at all follow-up visits for the intervention arms will record any changes in medication use since the previous encounter as well as adherence with study drug, ATT, and ART.

11.1.3 Physical exam:

A full physical exam at **screening** will record weight, height, and vital signs (temperature, pulse, blood pressure respiratory rate), and will include but not be limited to an oral exam (for presence of thrush), lymphatic exam, chest auscultation, abdominal exam (tenderness, organomegaly), skin exam (rash, cutaneous infection) and neurological exam for localizing signs or signs of peripheral neuropathy (light touch by Ipswich Touch Test ⁸⁶, gait). An interim targeted physical exam will be performed on all follow-up visits, including weight but not height and also including pulse oximetry. The screening exam of omics control group participants will be similar but will not include the neurological evaluations.

11.1.4 Counselling procedures:

All participants enrolled into the METHOD study will receive HIV counselling. Newly diagnosed participants who have not been initiated on ART will receive HIV counselling. Participants who are already on ART should be counseled on adherence to ART.

11.1.5 Radiological procedures:

A 1-view digital chest radiograph will be obtained on all enrolled participants at baseline and at week-24 (TB treatment completion). All chest x-rays are performed by the Radiology Department of Zamokuhle Hospital, Jose Pearson TB Hospital or Life Mercantile Hospital or Green Acres Hospital. The x-rays will be classified by METHOD trial physicians according to NTRDA criteria. The x-ray image files will be scrubbed of personal identifiers by the data management team at Tembisa and then sent to UMMS for grading by blinded, trained readers according to a validated numerical TB severity scale ⁶⁸ (see Section 6.1).

11.1.6 Questionnaires:

St. George's Respiratory Questionnaire (SGRQ) ^{62;63} will be administered at enrollment (V01) and repeated at week-12 (V11) and week-24 (V13).

11.1.7 Electrocardiogram:

A 12-lead electrocardiogram (ECG) will be obtained at enrollment (V01) to provide a baseline and to identify evidence of ischemic heart disease or arrhythmia.

11.1.8 Lung health assessment:

The **6-minute walk test** (6MWT) will be performed according to ATS standards ⁶¹ at enrollment (V01) and repeated at week-12 (V11) and week-24 (V13). Resting SpO₂ will be measured by **pulse oximetry** at rest in the seated position immediately before starting the 6MWT repeated immediately after participants reach the final 6MWT distance. The 6MWT is performed to identify differences in exercise capacity and overall cardiopulmonary function between the two study arms with results expressed as the 6MWT distance.

Pulse oximetry is performed to identify any difference in longitudinal trend of gas exchange between the two study arms. Exercise oximetry is measured at enrollment (V01) and repeated at week-12 (V11) and week-24 (V13) in conjunction with the 6MWT to calculate the distance-saturation product ⁸⁷. Resting pulse oximetry is performed with the physical exam done on every follow-up visit.

Spirometry using an EasyOne Pro instrument will be performed according to ATS/ERS standards ⁴⁰ at enrollment (V01) and repeated at week-12 (V11) and week-24 (V13) to identify any differences between study arms reflecting obstructive and/or restrictive ventilatory defects.

Pulmonary function assessment will not be performed on omics control group participants.

11.2 Laboratory Evaluations

11.2.1 Clinical Laboratory Services

The Aurum Institute's BRL Tembisa Laboratory, Ampath and National Health Laboratory Service will conduct all TB testing, SARS-CoV-2 testing, and other POC tests including glucometry on a scheduled basis and POC lactate tests as needed if participants present with signs or symptoms suggestive of LA. Safety laboratory tests will be performed by **Cytespace Africa Laboratories** (Cytespace Labs; <https://cytespacelabs.com/> and Ampath and National Health Laboratory Service). The Cytespace and Ampath are privately owned central and reference laboratories specializing in clinical trials in the clinical research industry. They support a wide spectrum of clients including research and development, biopharmaceutical, medical device and diagnostics customers. They have vast experience providing laboratory solutions for clinical studies. Clinical trial samples will be transported daily from the Tembisa CRC and Isango Lethemba TB Research Unit to Cytespace, Ampath and National Health Laboratory Service Laboratories.

11.2.2 Clinical chemistries:

Electrolytes (Na, K, Cl, CO₂) are measured at screening to check exclusion criteria and repeated at study weeks 4, 8 and 16. Electrolytes may also be measured at any scheduled or unscheduled follow-up visit for participants having signs or symptoms suggestive of LA. Study drug would be held and further evaluations would be pursued, according to protocol, for bicarbonate <18 mmol/L. Electrolytes for the omics control group are measured only at the screening visit to check exclusion criteria.

Creatinine is measured at screening to check exclusion criteria and repeated at week-8 and week-16 to screen for acute kidney injury during the study. Additional creatinine measurements would be performed on any participant who receives intravenous radiographic contrast between screening and week-12, corresponding to the duration of study drug treatment. The creatinine and anthropometric values are used to calculate the eGFR by the CKD-EPI equation, which provides a more accurate estimate than other commonly used estimating equations for individuals with normal or near-normal GFR (>60 mL/min/ 1.73 m²)⁸⁸. This equation was developed using pooled data from ten studies and validated against sixteen additional studies that used direct measurement of GFR by external filtration markers as the gold standard. It has been validated in African-Americans⁸⁸ and in black South Africans⁸⁹. Renal dysfunction defined as eGFR <60 mL/min/1.73m² is an exclusion criterion at screening. Study drug treatment will be stopped in any participant who develops sustained renal dysfunction after screening. This rationale for this exclusion is the potential for renal dysfunction to increase the risk for MALA, which was reported to occur only with eGFR <30 mL/min/1.73m² in patients with type 2 DM^{90;91}. Creatinine is only measured in the omics control group at the screening visit to check exclusion criteria.

Fasting blood glucose (FBG) is tested at screening visit for all participants in the interventional study and the omics control group to address the exclusion criterion of DM. Candidates will be classified as diabetic by WHO criteria (FBG ≥7.0 mmol/L)⁹². Random **fingerstick blood glucose** (FSBG) is measured by POC glucometer (OneTouch Ultra, LifeScan) in metformin arm participants only when the study drug is started at week-1 and at all subsequent visits through the final AE assessment (week-16).

Lactate will be measured, as needed, in participants who report symptoms compatible with LA or who have serum bicarbonate <18 mmol/L on any scheduled blood test. This test will be performed

using a POC lactate instrument (Accutrend, Roche Diagnostics) that was validated for use in African HIV⁺ individuals ^{93;94}. Study drug will be held for lactate >4.4 mmol/L (i.e. 2X ULN). Study drug may be resumed, per protocol, depending on resolution and the presence or absence of reversible risk factors for LA.

Hepatic function panel (HFP) includes alanine transaminase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total and direct bilirubin. The HFP is tested at screening to check exclusion criteria, repeated at week-8 to check for hepatotoxicity on ATT and again at week-16 as part of the AE assessment. The rationale is to exclude participants with hepatic dysfunction, which might increase the risk for MALA ⁹⁰ although that association has been questioned ⁹². A trial testing whether metformin has a protective effect on liver fibrosis in HIV/HCV co-infected participants is underway ⁹⁶. In another study, metformin was found to be safe for use by diabetic patients with hepatic cirrhosis ⁹⁷. Candidates with ALT or AST >3X the ULN at screening will be excluded from participation. Study drug and ATT will be stopped if enrolled participants develop signs or symptoms compatible with drug-induced liver injury and have ALT or AST >3X ULN, or if they have ALT or AST >5X ULN without symptoms. The hepatic function panel is tested in both the treatment arms and omics control group.

11.2.3 Hematology, Immunology and Virology

Complete blood count is obtained at screening to check the exclusion criterion of moderate to severe anemia that might otherwise provoke symptoms suggesting LA. It is repeated at week-8 and week-16 to monitor for hematological AEs of metformin. Complete blood count will be obtained for both the treatment arms and omics control group.

HIV serology results will be obtained from the medical records of potential METHOD trial participants during screening. Only participants who are newly diagnosed with HIV will be tested at the relevant site to confirm the HIV status using the Uni-Gold and Determine test strip POC tests. If both POC tests are positive, participants will be classified as HIV⁺. If there is disagreement between the two POC tests, then laboratory-based HIVserotesting will be performed by the Cystespace or Ampath. . HIV-seropositivity is an inclusion criterion for the METHOD trial. HIV serology results will be obtained for both the treatment arms and omics control group.

Hepatitis B surface antigen is tested at screening to check exclusion criteria intended to reduce risk factors for MALA in the METHOD trial cohort. This test will be obtained for both the treatment arms and omics control group.

HIV viral load is measured at enrollment as a baseline level for comparison and repeated at week-16 to identify any influence of metformin on the efficacy of ART. HIV viral load will be obtained for the omics control group only at enrollment.

CD4+ T-cell count is obtained at screening to check the exclusion criterion of <50 cells/mm³ that was selected to avoid enrolling unstable participants who might be at greater risk for potential AEs of metformin. The CD4+ T cell count is repeated at week-8 to assess potential HDT activity against HIV. This test will be obtained for both the treatment arms and omics control group.

COVID-19 molecular testing is performed at screening on all candidates using a rapid antigen test..

11.2.4 Microbiology

Sputum for Xpert MTB/RIF or ULTRA assay for all participants at screening will be performed by the Aurum Institute's BRL Tembisa Laboratory and Ampath. To increase sensitivity, only those participants Ct<25 will be invited for study participation. All candidates for the omics group will have Xpert MTB/RIF testing and those with positive results will be excluded.

Sputum for Mtb culture is obtained in all participants for the treatment arms at enrollment as the baseline and then repeated at every follow-up visit for the treatment arms. Sputum Mtb culture will only be tested in the omics group if there is clinical suspicion for active TB based on chest x-ray or symptoms despite a negative Xpert result. Liquid (MGIT) cultures are used to test the microbiological outcome of time to conversion and to support pharmacokinetic-pharmacodynamic (PK-PD) modeling. Solid culture (Lowenstein-Jensen media) is used to test the complementary microbiological outcome of month-2 culture status because there is a wealth of data linking this to the outcomes of failure and relapse⁹⁸ with no comparable data for liquid culture. All Cultures will be performed by the Aurum Institute's BRL Tembisa Laboratory and Ampath. Cytespace Laboratories will serve as a back-up laboratory for TB testing.

11.2.5 Whole blood bactericidal activity

Whole blood bactericidal activity (WBA) is applicable for the treatment arms only and not the omics control group. WBA is a candidate biomarker for assessment of protective antimycobacterial immunity and chemotherapy⁹⁹. Samples for WBA will be collected at week-5 at the Aurum Tembisa CRC site and Isango Lethemba TB Research Unit from a maximum of N=40 participants taking DTG, in the same time points corresponding to sparse PK sampling. Cultures consist of equal 300 µl volumes of heparinized blood and tissue culture medium, to which *Mtb* is added. Essentially all bacilli are quickly ingested by neutrophils and monocytes. After a 4-day incubation, bacilli are recovered, and the extent of growth or killing determined by inoculation into MGIT and monitoring time to positivity. Results are expressed as log change in viability, with positive values indicating growth. Numbers of cells (including neutrophils) and concentrations of drugs and other soluble antimicrobial mediators in whole blood cultures reflect those in vivo at the time of phlebotomy.

Evidence supporting a role for measurement of WBA in TB HDT trials comes from a recent study of 4 HDT candidates conducted by Dr Wallis. Experimental treatments (auranofin, CC-11050, everolimus, and ergocalciferol) were given for the first 112 days of rifabutin-substituted standard therapy. WBA was measured on three study days in the trial: day 42 (during the intensive phase), day 84 (during the continuation phase), and day 140 (late in the continuation phase, after experimental treatments had ceased). Mean values for each treatment arm at 0-8 hour post-dose on day 84 appear in Figure 1. In the absence of TB chemotherapy, *M. tuberculosis* H37Rv typically shows 0.2 log/day growth in whole blood culture. As Figure 1 indicates, killing, rather than growth, is apparent throughout the dosing interval in all study arms; this may be attributable to the 45 hour plasma half-life of rifabutin. Maximum effects (ie, minimum values) were apparent 3-4 hours post dose, also consistent with rifabutin pharmacokinetics.

Summary measures of mycobactericidal activity (maximal and total effects throughout the dosing interval) appear in Table 1. Only auranofin showed superior activity compared to control. This was most evident on day 84, and most evident in its maximum effect. The effect of auranofin was lost by day 140, 5 weeks after its discontinuation.

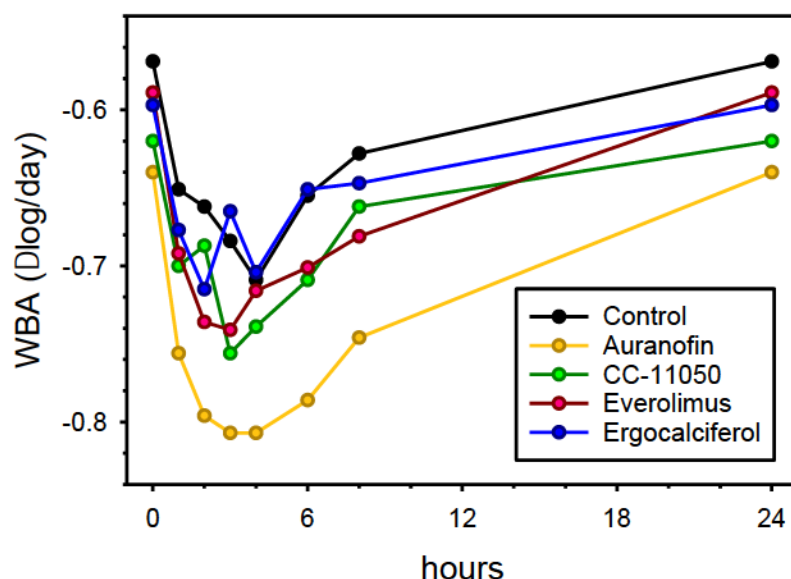


Figure 1. Mean whole blood bactericidal activity (WBA) by treatment arm, on day 84. The curve for CC-11050 does not include a potential effect of the second daily drug dose.

Table 1. Whole blood bactericidal activity (WBA).

	Day 42				Day 84				Day 140			
	WBA	Difference from control			WBA	Difference from control			WBA	Difference from control		
	Mean	Mean	95% CI	P	Mean	Mean	95% CI	P	Mean	Mean	95% CI	P
Total effect per dose ($\Delta\log$)												
Control	-0.627	0			-0.619	0			-0.642	0		
CC-11050	-0.669	-0.042	-0.153; 0.069	0.45	-0.662	-0.043	-0.148; 0.062	0.41	-0.57	0.071	-0.023; 0.167	0.13
Everolimus	-0.625	0.002	-0.112; 0.115	0.97	-0.657	-0.038	-0.130; 0.053	0.41	-0.657	-0.015	-0.108; 0.077	0.74
Auranofin	-0.702	-0.075	-0.186; 0.053	0.17	-0.72	-0.101	-0.198; -0.005	0.03	-0.621	0.020	-0.083; 0.125	0.69
Ergocalciferol	-0.66	-0.033	-0.131; 0.064	0.49	-0.638	-0.019	-0.123; 0.085	0.71	-0.696	-0.053	-0.136; 0.029	0.19
Maximal effect ($\Delta\log/\text{day}$)												
Control	-0.754	0			-0.766	0			-0.787	0		
CC-11050	-0.82	-0.065	-0.172; 0.042	0.22	-0.817	-0.050	-0.158; 0.057	0.35	-0.758	0.029	-0.71; 0.131	0.56
Everolimus	-0.815	-0.060	-0.168; 0.048	0.26	-0.836	-0.070	-0.153; 0.012	0.09	-0.828	-0.041	-0.144; 0.062	0.43
Auranofin	-0.891	-0.136	-0.256; -0.17	0.02	-0.895	-0.129	-0.223; -0.034	<0.01	-0.789	-0.001	-0.108; 0.107	0.90
Ergocalciferol	-0.816	-0.061	-0.167; 0.045	0.25	-0.804	-0.037	-0.151; 0.076	0.5	-0.857	-0.069	-0.156; 0.018	0.12

*Negative values indicate killing. Values for CC-11050 may underestimate effects as the period of sampling (0-8 hrs) did not include the second dose.

11.2.6 Pharmacokinetics

Pharmacokinetics is applicable for the treatment arms only and not the omics control group. Intensive and sparse sampling for PK-PD analysis of metformin toxicity and efficacy is performed at week-5. Participants enrolled at the Aurum Tembisa CRC in the treatment arms who consent to the additional intensive PK-PD testing and who are treated with DTG will be assayed at 0, 1, 2, 4, 6, 8, 10 and for metformin (metformin arm only, n=10), isoniazid (both arms, n=20) rifampicin (both arms, n=20), and pyrazinamide (both arms, n=20) at the Aurum Pretoria CRC. Population PK models will be used to describe the exposure of metformin and the

first-line ATT agents. The concentration-time data will be interpreted using nonlinear mixed-effects modelling with NONMEM 7.3 software.

11.2.7 Research Samples

Phlebotomy for research samples is performed at enrollment, week-8 and week-24 on the interventional arm participants and they will be performed at the Aurum Tembisa CRC site only. A single phlebotomy for research samples is performed at enrollment on the control participants. PBMC, plasma, serum, RNA and DNA will be isolated from blood samples and stored on site at the Aurum Tembisa CRC. Samples may be stored for up to 5 years for future cytometry, proteomic and transcriptomic studies as determined by results from the initially evaluated samples and by available funding.

11.2.8 Specimen Preparation, Handling and Shipping

Specimens that require preparative handling and labeling for storage at the Aurum Tembisa CRC only (aliquots of plasma, frozen PMBC, RNA storage tubes) will be processed in the research laboratory on site by a Laboratory Technician.

Specimens for clinical laboratory tests will be collected at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit by drivers employed by Cytespace, NHLS and Ampath.

Specimens for the research laboratory at SIgN will be prepared and shipped as specified in Aurum's SOP for Specimen Shipping Instructions.

11.2.9 Biohazard Containment

Transmission of HIV and other bloodborne pathogens can occur through contact with contaminated needles, blood, and blood products. Respiratory pathogens such Mtb are transmitted by inhalation. Appropriate blood, secretion, and respiratory precautions will be employed by all personnel in the collection of clinical samples and the shipping and handling of all clinical samples and isolates for this study, as currently recommended by the Centers for Disease Control and Prevention in the United States, the WHO internationally and the National Institutes of Health.

All protocol specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates will be shipped as specified for UN 2814 Category A Infectious Substances.

11.2.10 Total Blood Volume

Blood is collected in NaF, EDTA, SST and heparinized tubes. Some clinical laboratory tests can be performed on a single blood tube (e.g., serum electrolytes, creatinine, and hepatic function panel from a single 2.5 mL SST tube). CBC and CD4 count require 2 mL each. HBsAg requires 5 mL. Samples for PK-PD are 2 mL at each time point with an additional 2 mL sample at three of those time points for whole blood bactericidal assay (WBA). Research lab tests require 20 mL for flow cytometry, 5 mL for RNA and 4 mL for plasma (total 29 mL). The volume of blood drawn at each visit is noted in Appendix B, Table 1 (Schedule of Procedures/Investigations) and in Sections 12.3.2.-11.3.4 below. The maximum volume of blood collected at any single visit is 38.5 mL, which

conforms to local EC guidelines for good clinical practices (GCP) in the conduct of research involving adult participants.

11.3 Schedule of Procedures/Evaluations: Timing and Definitions

The schedule of visits and evaluations is presented in Appendix B, Table 1.

11.3.1 Screening

The following **screening evaluations** performed the initial visit (**V00**) will determine whether candidates are eligible for enrollment in the METHOD trial. Candidates will be screened in person at the Tembisa CRC and Isango Lethemba TB Research Unit within a maximum of 14 days of identification through recruiting channels. The procedures that will be conducted during the screening visit is as follows:

1. Registration for the visit.
2. Administer informed consent forms.
3. Study ID assignment
4. POC HIV test for participants who are newly diagnosed with HIV and not on ART
5. Sputum Xpert MTB/RIF test.
6. Urine hCG for women of childbearing age.
7. Interview for screening evaluation including assessment of medical history and behavioral inclusion/exclusion criteria recorded by CRF.
8. Physical exam, recording height and weight for BMI determination.
9. Phlebotomy for screening labs listed in Appendix B, Table 1.
10. Chest x-ray.
11. SARS-CoV-2 molecular test.
12. Assess eligibility

Decision points for exclusion during the screening visit include steps 2 and 4 through 11 according to criteria listed Section 9.1. Candidates passing steps 2 and 4 through 7 will undergo phlebotomy for screening laboratory evaluations, chest x-ray, and SARS-CoV-2 testing. Qualifying candidates will be given an appointment for an enrollment visit within 48-72 hours. Candidates who decline participation in screening or who are excluded at the screening visit prior to phlebotomy (behavioral and medical history, weight) will receive counselling and referral to appropriate medical providers for management of TB and HIV.

All candidates will be screened for COVID-19 symptoms, contact history and recent activities with increased risk for exposure at step 6. Candidates that present with a confirmed COVID-19 diagnosis are ineligible for participation. Those endorsing symptoms leading to a high suspicion of COVID-19 will be excluded from further screening or participation but will be offered a confirmatory molecular test. Since the symptoms of TB and COVID-19 may overlap, suspicion of TB/COVID-19 co-infection will be based on rapid onset of new symptoms and/or sudden worsening of prior systems, close contact with someone known to have COVID-19, participation in group gatherings with more than 10 individuals (funerals, conferences, sporting event etc.), and/or have been to an area with high rates of COVID-19.

The total blood volume taken at screening is 15.5 mL.

11.3.2 Enrollment

The following **enrollment evaluations** will be performed on **V01** at the Tembisa CRC and Isango Lethemba TB Research Unit in this stepwise order:

1. Review of screening labs. (Treatment arms and omics control group)
2. Randomization.
3. ECG. (Treatment arms)
4. Weight, vital signs, and physical exam. (Treatment arms and omics control group)
5. Interview for enrollment CRF completion. (Treatment arms and omics control group)
6. Phlebotomy for viral load and research labs. (Treatment arms and omics control group)
7. Sputum culture (liquid and solid media). (Treatment arms and omics control group)
8. SGRQ. (Treatment arms only)
9. Spirometry. (Treatment arms only)
10. 6MWT with oximetry. (Treatment arms only)
11. **Start ATT** after counselling for use and symptoms of potential AEs associated RHZE (or continue ATT if it was initiated prior to V01). (Treatment arms only)
12. Continue ART if started prior to screening (V00). (Treatment arms only)

Candidates may be excluded prior to randomization at step 1 listed above, based on screening laboratory results (Section 10.1.2) or if they report the initiation of ATT >14 days prior to V01. The enrollment CRF will collect detailed demographics, behavioral and medical history and current medications not captured at screening. Viral load, research labs, chest-ray, SGRQ and 6MWT with oximetry are performed to produce baseline values for comparison to later time points.

The total blood volume for enrollment studies is 32.0 mL.

11.3.3 HIV Counselling and Testing

All participants enrolled into the METHOD study will receive HIV counselling. Newly diagnosed participants who have not been initiated on ART should receive HIV counselling and testing at the screening visit. Participants who are already on ART should be counseled on adherence to ART.

11.3.4 Follow-Up

Follow-up evaluations will be obtained for the treatment arms in the order indicated below. Individuals in the omics control group end study participation when all evaluations listed in 11.3.2 are completed. Staff will follow-up with omics control group participants, as needed, to assess the need for any referrals based on blood, sputum, or chest x-ray results.

Visits that are one week apart (V02-09) will have a window of ± 2 days. Visits that are two weeks apart (V10-12) will have a window of ± 4 days. Visits that are one month apart (V11-12) will have a window of ± 7 days and visits that are two months apart (V13 and telephone follow-up at week 36) will have a window of ± 14 days.

The initial administration of the study agent for the metformin arm occurs on V02. The conditions for metformin to be started are the absence of all exclusion criteria based on medical history and screening lab test results. At all visits, follow-up CRFs include questions about interval medical

history, compliance with medications and review of systems, and questions focused on potential minor and major adverse events. The procedures and evaluations for the treatment arms of the study are as follows, where metformin use applies only that one arm with the other arm receiving standard ATT without metformin:

Week 1 (V02):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. **Start metformin 500 mg, 1 tablet daily** after counselling for proper use and symptoms of potential AEs.
6. Continue ATT (and ART if begun prior to this visit).

Venipuncture is not performed at V02.

Week 2 (V03):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. **Increase metformin to 500 mg twice daily.**
6. Continue ATT (and ART if begun prior to this visit). On this and any subsequent visits, participants may undergo additional evaluation for signs or symptoms of potential AEs. These evaluations would include a comprehensive physical exam and additional blood tests (venous pH, lactate, CBC).

Venipuncture is not performed at V03.

Week 3 (V04):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. Continue metformin 500 mg twice daily.
6. Continue ATT (and ART if begun prior to this visit).

Venipuncture is not performed at V04.

Week 4 (V05):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Phlebotomy for chemistries (electrolytes, creatinine, hepatic function panel).
5. Sputum culture (liquid media).
6. Urine hCG for women of childbearing age.
7. Continue metformin 500 mg twice daily.
8. Continue ATT (and ART if begun prior to this visit).

The blood volume for V05 is 2.5 mL.

Week 5 (V06):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Phlebotomy for PK-PD and WBA studies. Twenty consenting participants will be transferred to the Pretoria CRC for intensive PK sampling with collection at 0, 1, 2, 4, 6, 8 and 10 hours. Sparse PK sampling will be conducted on the maximum of 92 participants for PK-PD (0, 2 and 6 hours). The maximum of forty consenting participants in the sparse sampling group will have an additional 2.0 mL of blood collected for WBA at the same three time points (0, 2 and 6 hours).
5. Sputum culture.
6. Continue metformin 500 mg twice daily.
7. Continue ATT (and ART if begun prior to enrollment).

The total blood volume for V06 is 14.0 mL for the intensive PK sampling subgroup, 6.0 mL for the sparse sampling subgroup and 12.0 mL for the sparse sampling plus WBA subgroup.

Week 6 (V07):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
- 5.
6. Continue metformin 500 mg twice daily.
7. Continue ATT (and ART if begun prior to this visit).

Venipuncture is not performed at V07.

Week 7 (V08):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. Continue metformin 500 mg twice daily.
6. Continue ATT (and ART if begun prior to this visit).

Venipuncture is not performed at V08.

Week 8 (V09):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Phlebotomy for chemistries (electrolytes, creatinine, hepatic function panel), CBC, CD4+ T cells and research samples.
5. Urine hCG for women of childbearing age.
6. Sputum culture (liquid and solid media).
7. Continue metformin (one arm only), ATT and ART (**start ART** no later than this visit on all participants).

The total blood volume for V09 is 35.5 mL.

Week 10 (V10):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. Continue metformin (one arm only), ATT and ART.

Venipuncture is not performed at V10.

Week 12 (V11):

1. Follow-up CRF.
2. Weight, vital signs, oximetry, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Sputum culture (liquid media).
5. Urine hCG for women of childbearing age.
6. SGRQ, 6MWT with oximetry, spirometry.
7. **Stop metformin.**
8. Continue ATT and ART.

Venipuncture is not performed at V11.

Week 16 (V12):

1. Follow-up and AE Assessment CRFs.
2. Weight, vital signs, oximetry, and physical exam.
3. FSBG (metformin arm only).
4. Phlebotomy for chemistries, CBC.
5. Phlebotomy for viral load only on all participants.
6. Sputum culture (liquid media).
7. Urine hCG for women of childbearing age.
8. Continue ATT and ART.

The total blood volume for V12 is 7.5 mL

11.3.5 Early Termination Visit

Participants in the treatment arms and omics control group may withdraw study participation voluntarily at any time and/or they may be withdrawn involuntarily based on exclusions that are detected or newly develop after enrollment (e.g., negative baseline sputum culture or pregnancy). For the treatment arms, when possible, and with agreement by the participant, an early withdrawal visit will be arranged to complete any final evaluations as indicated depending on the study time point and to document the participant's early withdrawal from study follow-up. These evaluations will include interim medical history, targeted physical examination, and oximetry. The FSBG will be checked unless study participation is terminated before V02 or after V11. Other evaluations including creatinine, hepatic function tests, viral load or CD4+ T cells and research samples may be collected, depending on when termination occurs in relation to the scheduled evaluations (Study Schema, Appendix A; and Appendix B, Table 1). Lung health assessment (spirometry, 6MWT, SGRQ) will be performed if not done as a scheduled event within 2 weeks.

If participants miss a scheduled appointment, three attempts are made to contact them before that visit is declared as missed visit (Section 9.3). Participants will be withdrawn if they miss three

consecutive visits with documentation of three attempts to contact them for each of those missed visits.

If participation is terminated due to AEs, willing participants will be followed at scheduled intervals until the condition is resolved or becomes stable.

11.3.6 Pregnancy

Pregnancy at screening is an exclusion criterion for participation in both the treatment arms and the omics control group. This will be determined in women of childbearing age by urine hCG test at screening.

In the treatment arms, pregnancy poses an increased risk for and severity of MALA that would threaten the lives of the mother and the fetus¹¹⁰. Moreover, metformin might cause loss of appetite and weight loss in pregnant females that could adversely affect fetal development. In the omics control group, pregnancy would profoundly alter numerous transcriptomic and proteomic variables that would hamper analysis.

Metformin may also pose reproductive risks. A recent study used the Denmark national health registry to examine pregnancy outcomes associated with diabetes mellitus over a 20-year period (Wensink MJ, *Ann Int Med* 2022). Maternal diabetes was excluded to focus on effects of diabetes treatment on the male reproductive system. The main study finding was that the adjusted risks of an offspring birth defect for insulin, metformin, and sulfonylurea use, respectively, were 0.92 (95%CI=0.77–1.08), 1.40 (1.02–1.91), and 1.33 (0.83–2.12) relative to untreated diabetics. More specifically, metformin use during the period of spermatogenesis (within 3 months prior to conception) was associated with approximately a 3-fold increased risk of testicular non-descent or hypospadias. There was no excess risk if metformin exposure occurred outside the 3-month window. The authors acknowledge uncertainties regarding these findings, particularly regarding the numerically similar odds ratio due to sulfonylurea exposure. The small number of subjects in this group (less than half that of metformin) may explain its wide confidence interval; if so, some other biologic mechanism, involving glycemic control, may be invoked to explain similar findings for two different drug classes. This may increase uncertainty regarding the applicability of the findings to the men enrolled in this study, in whom diabetes is excluded. Nonetheless, to mitigate this potential risk, this study will extend the provision of effective contraceptive methods (described below) to female sexual partners of male study participants.

Pregnancy at screening is an exclusion criterion at enrollment and would prompt early termination from the METHOD trial if it is detected after enrollment and before week-16 (V13), which spans the period of study drug exposure plus 4 weeks after study drug is stopped. Use of contraception is a requirement for enrollment for women of childbearing potential. Women are considered post-menopausal or not of reproductive potential if they have not menstruated for at least 12 consecutive months (in the absence of medication known to induce amenorrhea) and have a documented follicle-stimulating hormone (FSH) level greater than 40 mIU/mL or a result in the testing laboratory's menopausal range. If an FSH level is not available, then women are considered not of reproductive potential if they have had 24 consecutive months of amenorrhea (in the absence of medications known to induce amenorrhea). For women of childbearing potential, the acceptable forms of contraception for the duration of study participation are:

Male or female condoms or diaphragms or cervical cap; PLUS one of the following methods:

- subdermal implant
- intrauterine device or intrauterine system

- combined estrogen and progesterone oral contraceptive
- injectable progesterone
- contraceptive vaginal ring
- percutaneous contraceptive patch

Contraception is not required if a woman:

- has reached menopause, with no menstrual period for one plus FSH level in the testing laboratory's menopausal range, OR no menstrual period for two years
- is documented to have had a hysterectomy
- is documented to have had bilateral oophorectomy
- is documented to have had tubal ligation

Adherence will be assessed, and counselling reinforced on every follow-up visit. Women of childbearing age who are not using intrauterine devices (IUDs) OR any other form of effective hormonal contraceptives prior to screening will be offered IUD placement at no cost. The use of IUDs is based on the need for immediately effective contraception since the interval between screening and first exposure to study drug will be no longer than 2 weeks. Women who choose the IUD method of contraception will be offered the option for removal of the device at completion or withdrawal from the study at no cost. Those who wish to continue using the IUD after the study will be referred to appropriate medical providers for long term follow-up.

Pregnancy tests will be done at screening, week 4, week 8, week 12, week 16 and week 24 in the study. If pregnancy is detected at any time point, it will be recorded and reported to the Regulatory Authorities in a pregnancy notification form. The participant will be followed to parturition to determine whether congenital anomalies are present. The package insert for metformin indicates that the drug was not teratogenic in rats or rabbits at doses up to 600 mg/kg/day, corresponding to an exposure of 2 and 6 times the maximum recommended human daily dose of 2550 mg based on body surface area comparisons of rats and rabbits, respectively. The placental barrier prevents transfer of metformin to the fetus and metformin was reported to be effective and safe for mother and fetus in gestational diabetes management trials¹¹¹. Because animal reproduction studies are not reliably predictive of human responses, the metformin package insert states that the drug should not routinely be used in pregnancy.

If a METHOD trial participant becomes pregnant, the study drug will be permanently discontinued but the participant will remain on study but off intervention. The occurrence of pregnancy will be reported to the registry and the participant will be followed for pregnancy outcomes.

11.3.7 Other Visits

Unscheduled visits can be initiated by either patient or provider and will be recorded on the Unscheduled Visit CRF. Participants will be counseled to contact the Aurum Tembisa CRC and Isango Lethemba TB Research Unit and return for unscheduled visits to assess any symptoms that could reflect AEs or if there is any change in medical condition or concomitant medications that could impact safety and continued participation in the trial. If symptoms of LA develop, participants will be instructed to report to the Zamokhule Hospital for the Aurum Tembisa CRC and participants will be instructed to report to the Livingstone Hospital or Netcare Greenacres hospital for the Isango Lethemba TB Research Unit site where they will be managed accordingly. Information from such visits will be recorded and promptly reported.

11.3.8 Final Scheduled Follow-up Clinic Visit

Week 24 (V13):

1. Follow-up CRF and Final Study Assessment CRF.
1. Weight, vital signs, and physical exam.
2. Phlebotomy for research samples.
3. Urine hCG for women of childbearing age.
4. Sputum culture (liquid culture).
5. SGRQ, 6MWT with oximetry, spirometry.
6. Chest x-ray.
7. **Stop ATT.**
8. Continue ART.

The total blood volume for V13 is 29.0 mL.

The combined total blood volume for all encounters over 6 months from screening through study completion is 136.0 mL for the twenty participants in the intensive PK sampling group, 134.0 mL for a maximum of forty participants in the sparse sampling plus WBA group, and 128.0 mL for a maximum of fifty-two participants who will have sparse PK sampling only.

11.3.9 Final AE Assessment

Week 36 (Telephone Follow-up):

1. Verbal AE assessment by CRF-guided phone contact.

Medical staff may request that participants return to the clinic for physical exam and laboratory or other evaluations based on participant responses to the telephone AE assessment.

12 ASSESSMENT OF SAFETY

12.1 Safety Assessment Overview

Risks relevant to the intervention and omics control groups include adverse reactions to phlebotomy and the radiation exposure from a single 1-view chest x-ray.

The intervention group will be taking metformin, which is the most widely prescribed drug used to treat DM worldwide and it has an excellent record of safety. Nonetheless, metformin carries a black box warning about LA, which is associated with mortality approaching 50% ¹¹². The black box warning in the FDA-approved metformin package insert states (in part): “Post marketing cases of metformin-associated lactic acidosis have resulted in death, hypothermia, hypotension, and resistant bradyarrhythmia. Symptoms included malaise, myalgias, respiratory distress, somnolence, and abdominal pain. Laboratory abnormalities included elevated blood lactate levels, anion gap acidosis, increased lactate/pyruvate ratio, and metformin plasma levels generally >5 mcg /mL.”

Despite this warning, the strength and even the existence of this association has been questioned. Some authorities hold that LA occurring in diabetic patients taking metformin is attributable to other factors commonly associated with diabetes (sepsis, dehydration, renal failure) ¹¹². A Cochrane review of 347 comparative trials and cohort studies with a combined 70,490 patient-years of metformin exposure identified no cases of fatal or non-fatal LA ⁷². Metformin exposure in the METHOD trial will total 11.8 patient-years.

The theoretical basis for MALA is from mitochondrial toxicity, which raises further concern that combined treatment with metformin and nucleoside reverse transcriptase inhibitors (NRTIs) with mitochondrial toxicity could compound the risk. While most previous clinical trials of metformin in HIV did not report instances of MALA, a trial of metformin combined with ART conducted in Botswana between 2002 and 2007 identified risk factors for the development of LA and symptomatic hyperlactatemia ¹¹³. In that study, participants received various combinations of zidovudine, lamivudine, didanosine, stavudine, zalcitabine, nevirapine or efavirenz. The major identified contributor to LA risk was stavudine that has been replaced by TDF, which has less toxicity. A finite risk for LA in METHOD trial participants certainly exists given case reports of LA occurring with TDF alone or combined with metformin ¹¹⁴⁻¹¹⁶. Lactic acidosis was also reported with concurrent use of DTG plus metformin ¹¹⁷. All of the individuals described in these case reports had other risk factors for LA (diabetes, sepsis) that will be absent from the METHOD cohort. We recognize that many individuals in the community with TB/HIV coinfection have risk factors for MALA but might benefit from metformin. In the absence of evidence that may be produced by the METHOD trial, the risk vs benefit for such individuals is unknown. If METHOD trial results support the safety of metformin given concurrently with ATT and ART, and if there is a signal of efficacy, then less stringent exclusions might be justified in a future phase 3 trial.

Based on available evidence, scheduled laboratory screening for LA would not contribute the safety of participants in the METHOD trial. Our approach to maximize safety is based on comprehensive counselling about the symptoms of LA and a plan of action if they occur. We will screen for conditions that have been identified as risk factors for MALA and exclude candidates with hepatic or renal dysfunction and those actively infected with HBV. Diabetic patients with those comorbidities are routinely treated with metformin in clinical practice but we believe our exclusion criteria are prudent given the SAE potential associated with metformin. Counselling will be reinforced at every study visit and participants will be advised to suspend the study drug if they develop sepsis, hypotension, renal failure, DM or undergo a radiological test with IV iodinated

contrast material. The study drug will be resumed in cases where these risk factors are transient and resolve but would be discontinued for non-resolving risks such as DM or chronic renal insufficiency.

Hypoglycemia is another potential SAE of metformin for participants in the METHOD trial, all of whom will be non-diabetic. The mechanism(s) of action for metformin are complex and unlike other classes of oral antidiabetic drugs. It reduces hepatic glucose production, opposes glucagon, decreases intestinal absorption of glucose, inhibits fatty acid synthesis, enhances beta-oxidation and it improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin does not provoke hypoglycemia in patients with type 2 DM unless there is concurrent use of other classes of anti-diabetic drugs. Metformin generally does not cause hypoglycaemia in normoglycemic individuals, except in special circumstances that could be present in some METHOD trial participants. Cho et al.¹¹⁸ reported that rifampicin enhanced the glucose-lowering effect of metformin in non-diabetic individuals evaluated by oral glucose tolerance test. The proposed mechanism of this effect is increased hepatic expression of OCT1, resulting in increased metformin delivery to the liver. Importantly, the combination of rifampicin plus metformin did not reduce baseline blood glucose values or cause hypoglycemia in that study and rifampicin exerts a countervailing effect by boosting intestinal glucose absorption¹¹⁹. Metformin-associated hypoglycemia can occur in when caloric intake is insufficient to meet the energy needs of strenuous physical exertion. We will minimize this risk in METHOD trial by excluding candidates with a history of DM, by counselling at enrollment and follow-up visits regarding the need to maintain adequate caloric intake, particularly with physical exertion, and counselling regarding the symptoms of hypoglycaemia and the actions to take if these symptoms occur.

Adverse effects expected to occur in >5% of METHOD trial participants are shown in Table 2. Gastrointestinal symptoms are expected to be the most commonly reported AE associated with metformin in METHOD trial participants. While typically mild and transient, GI toxicity is potentially the greatest barrier to the adoption of metformin as HDT for TB. Symptoms including nausea, vomiting, and/or diarrhoea typically occur during treatment initiation and in most cases improve spontaneously despite continued treatment and dose escalation. Persistent symptoms could result in dehydration, weight loss, and/or reduced adherence to treatment with the study drugs, ATT and/or ART. It is possible that GI toxicity could necessitate discontinuation of study drug in over 5% of METHOD trial participants. Gastrointestinal symptoms developing for the first time after an initial period of tolerated dosing would most likely be unrelated to metformin except if LA ensues. To minimize GI symptoms, participants will receive a starting dose of 500 mg⁵⁶.

Table 2. Adverse effects occurring in >5% of participants in a controlled trial of metformin monotherapy (up to 2,250 mg daily) vs placebo.

Symptom	Metformin (N=141)	Placebo (N=145)
Diarrhoea	53.2%	11.7%
Nausea/Vomiting	25.5%	8.3%
Flatulence	12.1%	5.5%
Asthenia	9.2%	5.5%
Indigestion	7.1%	4.1%
Abdominal discomfort	6.4%	4.8%
Headache	5.7%	4.8%

Source: Glucophage (metformin) package insert.

The following adverse reactions were reported in ≥1.0% to ≤5.0% of diabetic patients more commonly with metformin than placebo: abnormal stools, hypoglycemia, myalgia, lightheaded,

dyspnea, nail disorder, rash, increased sweating, taste disorder, chest discomfort, chills, flu syndrome, flushing and palpitation.

Adverse reactions rarely reported to occur in patients taking metformin include LA (discussed above), hypoglycemia (in the absence of any other anti-diabetic drugs), hepatic dysfunction, vitamin B12 insufficiency, anemia, peripheral neuropathy and drug allergy (manifesting as leukocytoclastic vasculitis or psoriasiform drug eruption).

12.2 Adverse Event Procedures and Reporting Requirements

12.2.1 General Considerations

Adverse events (AEs) of any severity are highly unlikely for omics control group participants but would be assessed and handled in a manner similar to that used for participants in the interventional arms.

International Conference on Harmonization (ICH) E6 defines an AE as any untoward medical occurrence in a clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product. The occurrence of an AE may come to the attention of METHOD trial personnel during follow-up visits and interviews with study participants using structure questionnaires or upon review by a study monitor, or in an unsolicited manner as reported by a participant outside of a scheduled visit. If the primary care team suspects an adverse event between study visits, this will trigger an unscheduled study visit to assess the potential AE.

All AEs will be captured on the Adverse Event CRF (AE001). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed by a METHOD trial study clinician), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to stability.

Any medical condition that is present at the time that the participant is screened and enrolled will be considered as baseline and not reported as an AE. However, deterioration of a preexisting medical condition during the study will be recorded as an AE.

All AEs will be graded for severity and relationship to study product.

A **serious adverse event** (SAE) is defined as meeting one of the following conditions:

1. Death during the period of protocol defined surveillance.
2. Life threatening event (defined as a participant at immediate risk of death at the time of the event).
3. An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance.
4. Results in congenital anomaly or birth defect.
5. Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical

intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

1. Recorded in a timely and proper manner and reported to the relevant entities.
2. Followed through resolution or stabilization by a study clinician
3. Reviewed and evaluated by a study clinician

The METHOD trial will use the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1, July 2017 (Appendix D). Any events that are not listed in this toxicity table will be graded by the local PI as shown in Table 3.

Table 3. Grading of adverse events

Grade	Severity	Characteristics
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate/Minimal	Local or noninvasive intervention indicated; limits age-appropriate instrumental activities of daily living (ADL)
3	Severe	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limits self-care ADL
4	Life-threatening	Urgent intervention indicated
5	Death	Related to AE

12.2.2 Expedited Reporting of Adverse Events Using the DAIDS Adverse Experience Reporting System (DAERS)

Because DAIDS will not be holding the IND for this study, DAERS will not be used.

12.2.3 Reporting Requirements for the METHOD Trial

The SAE Reporting Category, as defined in the DAIDS EAE Manual, will be used for this study. The study agent for which expedited reporting is required is metformin. In addition to the SAE Reporting Category identified above, other AEs that must be reported in an expedited manner include multi-organ system failure, renal failure and all hepatic failures.

The METHOD trial will report AEs to WHREC and SAHPRA as specified in SOP-IEC-005v6, Adverse Events Processing (Appendix E) and in the SAHPRA document Reporting Adverse Drug Reactions in South Africa version 1_2 (Appendix F), respectively. All suspected unexpected serious adverse reactions (SUSARs) will be reported to the DAIDS MO within 5 days of being reported to the site and quarterly cumulative SAEs will be sent to the DAIDS MO.

13 CLINICAL MANAGEMENT

13.1 Clinical Management of Adverse Events

We do not expect to encounter any AEs during the screening or enrollment visits for omics control group participants. Phlebotomy might be complicated by bleeding, hematoma formation or vasovagal syncope. Bleeding will be controlled with direct pressure until it stops, and the venipuncture site will be dressed. Risk of hematoma formation will be minimized by attention to proper phlebotomy technique and the prompt application of local pressure to prevent visible bleeding and hematoma expansion. Risks related to syncope, particularly falls, will be minimized by proper positioning and by close observation throughout the procedure. If syncope occurs, participants will be placed in the Trendelenburg position for recovery with monitoring of vital signs at least every 5 minutes until stable. Intravenous fluid resuscitation may be used based on the judgement of clinic medical staff. The range of other possible acute conditions that might arise during the screening or enrollment visits is too large to list additional management measures, but qualified medical staff are present on site at all times. Omics control group participants who experience any AE will be monitored in the clinic until stable or transported to hospital if indicated. For the intervention group, unanticipated and anticipated toxicities will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.

The AEs associated with metformin have almost exclusively been studied in the context of diabetes, which influences the rates at which these AEs are expected to occur. Common AEs anticipated in $\geq 5.0\%$ of diabetic people taking metformin include GI intolerance (manifesting as diarrhea, nausea/vomiting, flatulence, indigestion and/or abdominal discomfort), headache and asthenia. Uncommon AEs that may occur in 1.0-5.0% of diabetic people taking metformin include: abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, increased sweating, taste disorder, chest discomfort, chills, flu syndrome, flushing and palpitation. Participants in the METHOD trial will take ATT and ART as concomitant medications and have the potential to cause certain AEs that overlap with those associated with metformin. AEs assessed as related to concomitant medications or other non-study drugs will be handled according to the relevant package inserts and the best medical judgment of the site investigator.

13.1.1. GI Toxicity

It is anticipated that 10-20% of METHOD trial participants who receive metformin will experience mild GI symptoms of nausea, vomiting or diarrhea without right upper quadrant tenderness or transaminase elevation. Gastrointestinal symptoms typically occur soon after starting metformin treatment and abate spontaneously despite its continued use. The risk for GI symptoms will be minimized by dose escalation and by counselling participants to take the study drug with food and to take ATT on an empty stomach. If grade 1-2 symptoms develop during the first week of study drug treatment, the study drug will be continued. If these are GI symptoms, the participant will be advised that they are usually transient and likely to resolve despite continued use of the drug. If symptoms resolve spontaneously by the following visit, then the dose will be continued according to protocol. For grade 3 or higher symptoms of any kind, the study drug, ATT, and ART will all be stopped, and the participant will be reevaluated at 3-day intervals. If symptoms resolve by the first 3-day follow-up, then ATT will be resumed. If grade 3 symptoms do not recur after resumption of ATT, then ART will be resumed on the second 3-day follow-up. If grade 3 symptoms do not recur with resumption of ATT and ART, then study drug will be resumed on the third 3-day follow-up. This approach is used to identify the treatment most likely responsible for the symptoms. If the symptoms do not recur after restarting ATT and ART, then study drug would be suspected, and

this would be confirmed by re-challenge 9 days after stopping it. If grade 3 or higher symptoms recur when the study drug is resumed, then the participant would be classified as a study drug treatment failure due to AE. A similar approach will be taken for all grade 1-3 AEs. The clinical management of grade 1-3 toxicities potentially caused by metformin vs INH, RIF, PZA, EMB, TDF, FTC, and/or DTG or EFV is shown in Appendix B, Table 3. All grade 4 toxicities will cause permanent discontinuation of study drug.

Sections 13.1.2, and 13.1.3 are not relevant to the control arm of the trial.

13.1.2. Hyperlactatemia and Lactic Acidosis

Lactic acidosis is a rare SAE, occurring in approximately 0.03 cases/1000 patient-years, with approximately 0.015 fatal cases/1000 patient-years, according to the Glucophage (metformin) package insert. However, a Cochrane review of 70,490 patient-years of metformin exposure identified no cases of fatal or non-fatal LA ⁷².

Symptomatic hyperlactatemia (SH), a potentially serious toxicity that may be associated with metformin, is defined by one or more of specific symptoms (nausea/emesis, increased fatigue, dyspnea, muscle weakness, and/or paralysis of the lower extremities) along with serum lactate >4.4 mmol/L in the absence of acidosis. If participants report symptoms compatible with SH, then lactate will be measured by POC testing. If the lactate level is >4.4 mmol/L, then serum bicarbonate and venous pH will be evaluated on an urgent basis. If acidosis is excluded, participants will be managed by discontinuing study drug after confirming hemodynamic stability and reviewing potential causes of lactate elevation including sepsis, trauma, seizures, excessive muscle activity, regional ischemia, smoke inhalation, diabetic ketoacidosis, thiamine deficiency, liver dysfunction and other drugs associated with lactate elevation (acetaminophen, beta-2 agonists, NRTIs, linezolid, propofol, alcohols, theophylline, and cocaine). For grade 1-2 symptoms, participants will be advised to stop the study drug and return in 72 hours for repeat POC lactate testing that will be trended until <4.4 mmol/L.

Lactic acidosis is a potentially life-threatening toxicity potential associated with metformin and defined by venous pH <7.35 and serum bicarbonate <20 mmol/L. Participants who develop LA will undergo emergent management at Zamokhule Hospital and Jose Pearson TB Hospital. The precise interventions will be tailored to the requirements of individual participants in the best judgment of the physicians providing care. Management might include intravenous fluid resuscitation with isotonic sodium bicarbonate, hemodialysis to correct metabolic acidosis and eliminate metformin and endotracheal intubation with mechanical ventilation to compensate metabolic acidosis and maintain medical stability.

13.1.3. Reproductive Risks

A recent study using the Denmark National Health registry reported that preconception paternal metformin treatment was associated with genital birth defects in male offspring ¹⁴⁰. The risk was confined to metformin use during the period of spermatogenesis during 3 months before conception. To circumvent this risk, willingness to use condoms for the period of metformin use and 3 months after stopping metformin is an inclusion criterion and counselling will be reinforced at every visit. In addition to using condoms, males in the metformin arm will be asked to encourage their partners use a second form of contraception. Pregnancies in partners of male participants will be recorded using the Aurum Pregnancy Notification CRF, version 1.0.

13.1.4. Other Toxicities

Management of grade 1-3 AEs that are unrelated to the GI tract, elevated lactate, or listed in Appendix B, Table 3, will be managed by METHOD trial clinical staff under the direction of the Site PIs as needed according to their best clinical judgment and local practice norms. These AEs may include those listed on the metformin package insert (myalgia, lightheadedness, dyspnea, nail disorder, increased sweating, taste disorder, chest discomfort, chills, flushing and palpitation) or other symptoms that in the judgment of staff represent AEs. The general approach will be targeted relevant evaluation for a grades and discontinuation of study drug for 3-days for grade 2-3 AEs. In addition to a complete medical history and physical examination, targeted evaluations might include chest x-ray and oximetry (e.g., for breathlessness) or ECG (for palpitations), or any clinical test depending on the nature and severity of symptoms.

13.2 Other Disease Events

Other disease events that may occur in the METHOD trial cohort include complications of pulmonary or extrapulmonary TB, opportunistic infections related to HIV, common infections that may or may not be related to HIV such as community-acquired pneumonia. Because of effective malaria control for over 50 years in South Africa, the risk of malaria transmission is low, seasonal, and limited to the low-lying North-Eastern Provinces of KwaZulu-Natal, Mpumalanga and Limpopo¹²⁰.

Acute illness is an exclusion criterion for the METHOD trial. If such conditions are identified at screening or enrollment, acute management will be applied as indicated in the judgement of clinical staff, along with assessment for referral.

For the metformin arm, conditions that have potential for life-threatening complications could increase the risk of LA and result in premature study discontinuation for an individual participant (see Section 14.7.1). These conditions include:

1. Tuberculous meningitis (defined by lumbar puncture chemistries, cell count, PCR and culture)
2. Cryptococcal meningitis (defined by the presence of cryptococcal antigen in cerebrospinal fluid)
3. Septic shock (defined by mean arterial pressure ≤ 65 mmHg and lactate >2 mmol/L with defined site of infection, with or without bacteremia)
4. TB-IRIS

A recent meta-analysis estimated a summary risk for TB-IRIS in HIV⁺ individuals of ~18% with an attributable mortality of ~2%¹²¹. The syndrome presents in two forms, paradoxical and unmasking, based on the temporal relation to treatment of the underlying diseases. Paradoxical TB-IRIS manifests with new or recurrent TB signs or symptoms during ATT while unmasking TB-IRIS is characterized by hyper-inflammatory presentation of TB in HIV⁺ patients with early during ART¹²². The risk for TB-IRIS correlates with TB disease burden at the time of ATT initiation and with the degree of baseline immunosuppression in HIV⁺ individuals.

Manifestations of TB-IRIS may include suppurative lymphadenitis, splenic rupture, cold abscesses and worsening of dyspnea despite microbiologic response to ATT. Recent evidence suggests that lung inflammation during HIV-associated TB-IRIS results in PIAT¹²³. Owing to its anti-inflammatory properties, metformin has the potential to reduce the risk and/or severity of TB-

IRIS although this question has so far remained unexplored. For that reason, the occurrence of TB-IRIS is an exploratory endpoint for the METHOD trial (Section 14.2.3).

Public health clinic physicians have primary responsibility for managing HIV in METHOD trial participants. If Aurum Tembisa CRC and Isango Lethemba TB research Unit physicians suspect that cryptococcal or TB meningitis, septic shock or TB-IRIS has occurred in a participant, they will provide necessary initial care and facilitate a safe and appropriate hand-off to public health clinic staff.

Serious other disease events will prompt discontinuation of the study drug.

13.3 Pregnancy

Pregnancy monitored at screening is an exclusion criterion for enrollment in the METHOD trial. Pregnancy occurring between the time of enrollment and study drug completion for the metformin arm will result in discontinuation of study drug, but the participant will continue to be followed. This is not expected to occur since women of childbearing age must use effective contraception to be eligible for enrollment.

13.4 Breastfeeding/Replacement Feeding

Breastfeeding is an exclusion criterion for the METHOD trial.

Mean infant exposure to metformin was reported to be 0.28% of the weight-normalized maternal dose. While this is well below the 10% level of concern for breastfeeding in term of infant safety, there is a theoretical risk for hypoglycemia in the infant. The maternal caloric depletion associated with breastfeeding has a theoretical potential to precipitate LA in the mother. For these reasons, breastfeeding is an exclusion criterion for participation in the METHOD trial.

13.5 Treatment Failure

Treatment failure for TB is defined by positive culture at month-5 or month-6 of ATT or by the development of acquired drug resistance at any time during ATT. TB treatment failure is a secondary outcome measure for the METHOD trial but the management of treatment failure is not an objective of the protocol. Treatment failure will be monitored by monthly sputum culture during ATT. Participants in either treatment arm who fail standard ATT while participating in the METHOD trial will be referred to the local public health providers who have responsibility for TB treatment in Gauteng Province.

Treatment failure for HIV is defined by viral load >1000 copies/mL on two consecutive measurements after 3 months of ART, or by CD4+ T cell count falling to the pre-ART baseline. Viral load and CD4+ T cell count will be tested at baseline and at the completion of study drug treatment in METHOD trial participants to reveal any anti-HIV HDT activity of metformin, but the management of HIV treatment failure is not an objective of the protocol. HIV treatment failure will be managed by the local public health clinicians who will remain responsible for providing ART to METHOD trial participants.

13.6 Criteria for Discontinuation

13.6.1 Criteria for Permanent Intervention Discontinuation for an Individual Participant

The criteria for permanent discontinuation of metformin for an individual participant are:

1. Sputum Mtb culture resistant to isoniazid, rifampicin, or pyrazinamide at any time during study participation.
2. Grade 3 or higher study intervention-related AE lasting >7 days after stopping the intervention, or that improves off intervention but is judged by the investigator to have a high risk of recurring on re-challenge.
3. Requirement for prohibited concomitant medications.
4. Pregnancy or breastfeeding.
5. Grade 3 GI intolerance that persists after counselling for the optimal timing of ingestion and after stopping the intervention for 7 days or subsides after the intervention is stopped but recurs after re-challenge with the intervention.
6. Grade 3 or higher hypoglycemia.
7. Any grade 4 AE.
8. Weight loss >6% of baseline value.
9. Impaired renal function defined as eGFR <60.
10. Completion of study intervention as defined in the protocol.
11. Request by participant to terminate study intervention.
12. Clinical conditions that, in the best judgment of the investigator, may be harmful or potentially life-threatening to the participant, even if not addressed in the AE Management section of the protocol.
13. Recommended by the EC/IRB, DSMB, OHRP, in-country national health or regulatory entity.

The participant will continue to be followed, with his/her permission, if the study intervention is discontinued. No subsequent modifications to the visit schedule and duration of continued follow-up will be made, except for discontinuation of the study product.

13.6.2 Criteria for Premature Study Discontinuation for an Individual Participant

Participation in the METHOD trial would be discontinued during the screening or enrollment visit if candidates experienced an adverse event related to phlebotomy that, in the judgement of trial staff, precluded further attempts at phlebotomy. Participation would also be discontinued if candidates developed any acute illness that disqualified enrollment during the screening or enrollment visit. When possible, a safety assessment by telephone or in the clinic will be conducted at one month after discontinuation from the study.

The criteria for premature discontinuation from the study for an individual participant are:

1. Lost to follow up as evidenced by failure by the participant to attend 3 consecutive clinic visits, despite documented efforts by the retention team to locate and, if necessary, provide transport to the clinic, at the discretion of the site investigator.
2. Breastfeeding.
3. Request by participant to withdraw.
4. Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.

-
5. Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of study results.

14 STATISTICAL CONSIDERATIONS

We will follow the Consolidated Standards of Reporting Trials (CONSORT) 2010 to analyze and report the METHOD trial. A CONSORT 2010 Flow Diagram will be generated to report results of enrollment, randomization, follow-up/retention, and number of participants available for analysis. The CONSORT 2010 Flow Diagram as well as the Checklist will guide the preparation of interim and final trial reports, and regular reporting to the DSMB and progress report.

14.1 Overview and General Design Issues

TB remains pandemic and a leading cause of death in people living with HIV despite the availability of effective antimicrobial agents. Several barriers explain the failure to bring about a faster decline in TB incidence and prevalence, among them the current requirement for a minimum 6-month course of ATT to achieve cure. Even with curative treatment, PIAT occurs in half of all TB survivors, adversely affecting their capacity for work and placing them at risk for chronic, progressive respiratory complications including COPD and bronchiectasis. Preclinical studies have shown that adding metformin to ATT accelerates the elimination of Mtb infection and modulates immune pathology in the mouse aerosol TB models¹³.

Retrospective clinical data support the relevance of findings in mice to human TB patients. Fundamental studies identified cellular mechanisms associated with the HDT efficacy of metformin, including enhanced generation of mitochondrial ROS and enhanced activation of antigen-specific CD8+ T cells. This preclinical evidence provides a compelling scientific rationale to test metformin in a randomized, controlled trial in HIV-infected adults with pulmonary TB. There exists a wealth of safety data on metformin in HIV-infected and in diabetic patients, but it is presently unknown whether metformin is safe and tolerable when given concurrently with ATT and ART. In the absence of that knowledge, it is unfeasible to propose a definitive trial adequately powered for a definitive test of HDT efficacy. The METHOD trial is a Phase 2A trial focusing on safety but will also leverage the cohort for a preliminary evaluation of efficacy to inform the design of the subsequent Phase 3 trial. This study is designed as a two (parallel)-arm randomized, open-label trial with fixed size and equal probability for standard treatment vs. metformin added to standard treatment for each arm.

14.2 Study Endpoints

14.2.1 Primary Endpoint

The primary endpoint is the number of grade 3 or higher adverse events during the time on study drug plus 4 weeks post study drug completion.

14.2.2 Secondary Endpoints

Study endpoints are applicable only to the intervention group.

The secondary efficacy endpoints of the METHOD trial include:

1. Time to sputum liquid culture conversion.
2. Change in FEV1% from baseline to treatment completion.
3. Exercise capacity at treatment completion defined by the 6MWT distance and the distance-saturation product.
4. Change in radiographic severity score from baseline to treatment completion.

5. Respiratory quality of life quantified by the change in SGRQ from baseline to treatment completion.

The secondary safety endpoint of the METHOD trial is:

1. Proportion of participants experiencing one or more AE of any type and grade.

14.2.3 Exploratory Endpoints

1. HIV status defined by viral load and CD4+ T cell count.
2. Occurrence of TB-IRIS using an internationally accepted definition suitable for low-resource settings ¹²⁴.

14.3 Study Objectives and Hypothesis/Hypotheses

Study objectives and hypothesis/hypotheses are applicable only to the treatment arms of the study.

Study Objective 1. Determine the tolerability and safety of metformin added to standard ATT and ART in participants co-infected with HIV and TB.

Hypothesis: Metformin has acceptable tolerability and safety when combined with ATT and ART in patients co-infected with TB and HIV.

Statistical approach:

Metformin would be unsuitable as an HDT agent for TB/HIV co-infection if GI toxicity compels discontinuation due to unacceptable symptoms or safety concerns in more than 5% of cases. This will be tested as a two-arm non-inferiority trial with binary outcomes.

Average and median numbers of grade 3, 4 and 5 adverse events will be tabulated by arm, with and without stratification by sex, age and TB severity. Because the number of AEs may have a skewed distribution, we will use Poisson or negative binomial distribution-based tests to evaluate whether number of adverse events differs between arms (crude analysis). Poisson regression models will be used to compare rates of adverse events between the two arms, with and without stratification by sex, age, and TB severity. Adjustment for other unbalanced host factors will be considered in the models as needed. In case the adverse events are rare or existence of over-dispersion, negative binomial regression models will be used for analysis. Using these models, we will also attempt to identify host factors predictive of potential adverse events. In addition, similar analyses will be conducted for each type and grade of adverse events (e.g., intolerability). If adverse events are rare and typical statistical models cannot be used effectively for small numbers, we will enumerate the events, overall and by sex, age group and severity.

There is a chance that nearly all participants may hit the secondary safety endpoint of at least one AE of any grade. To address that possibility, we will consider analysis of the number of secondary AEs of any grade, instead of any vs. none. We propose to use either Poisson or negative binomial regression models to analyze incidence rates of secondary AEs. This approach will allow us to differentiate participants subject to risks for multiple AEs from those with risks to none or few AEs.

Study Objective 2. To evaluate the efficacy of metformin as adjunctive therapy when added to standard ATT and ART for TB/HIV co-infection.

Hypothesis: Metformin combined with standard ATT accelerates sputum conversion and improves respiratory outcomes of pulmonary TB disease in HIV coinfecting individuals.

Statistical approach: The efficacy of metformin at HDT for TB could manifest as accelerated sputum conversion and/or as enhanced respiratory health after TB treatment completion. This will be evaluated using several outcome measures including:

- Time to sputum conversion in MGIT culture
- Change in FEV1% from baseline to TB treatment completion
- Change in distance-saturation product from baseline to TB treatment completion
- Change in radiographic severity score from baseline to TB treatment completion
- Change in SGRQ score from baseline to TB treatment completion

Time to sputum conversion in MGIT culture will be analyzed and compared between the metformin and standard treatment arms using log-rank test (crude analysis) and Cox proportional hazards models with adjustment for relevant unbalanced covariates. In addition, heterogeneity in metformin treatment effects on time will be evaluated by stratifying the analysis by sex (male vs female), age group (<45 vs ≥45y) and TB severity (mild vs moderate vs severe). Further, multivariable Cox regression models will be used to examine differential treatment effects by these variables, or certain combinations of any of the three factors (interactions), adjusting for other relevant host factors (controlling for confounding). Interaction terms between treatment indicators (or MET dosage variable) and host factors (sex, age and severity) will be included in the models. Statistically significant interactions may indicate potential heterogeneous metformin effects by these host factors, and the need for in-depth analysis and reporting by subgroups. Such analyses will help answer clinical questions such as whether metformin effects will be stronger among or limited to certain patient subgroups. For other secondary outcomes including changes in FEV1%, FVC, FEV1/FVC, distance-saturation product, radiographic severity score and SGRQ score, we will apply linear and generalized linear regression models as appropriate. The analytic strategies are similar to those for time to sputum conversion, except the underlying statistical models. We are fully aware that the sample size of the trial is limited, and its statistical power is limited for testing some of these proposed subgroup analyses. These analyses are unlikely to be definitive but will be helpful to generate hypotheses and inform the design of the subsequent Phase 3 trial.

Study Objective 3. Determine whether metformin added to standard ATT in participants co-infected with and treated for HIV improves HIV outcomes.

Hypothesis: By activating AMPK and by modulating inflammatory cytokine expression, metformin enhances the efficacy of ART.

Statistical approach: This exploratory objective is investigated using the endpoints of viral load and CD4+ T cell count, and the occurrence of TB-IRIS. As viral load and CD4+ T cell counts usually have skewed distribution, we will consider logarithmic transformation and use of quantile regression models. Quantile regression models will allow us to evaluate the metformin effect on changes in distributional characteristics of viral load and CD4 counts (e.g., significant decreases in lower quartile or median values of viral loads and increases in median and upper quartile values of CD4+ counts when treated with metformin). Statistical comparisons are very similar to those for Study Objective 3, except the outcomes and the underlying statistical models (and thus not repeated).

General statistical considerations applicable to all study objectives: In these analyses, model performance, distributional assumptions and functional forms will be carefully examined. To address issues regarding participant dropout, we will compare characteristics of the completers and the dropouts. If dropout is prevalent (e.g., >10%), we will build a logistic model to examine whether the dropouts are related to treatment arm and host factors. If dropout rates are relatively high and data can support, we will use the model to estimate the probability of dropout and use the probability for inverse probability weighting in the final analysis (also called marginal structural equation models). Dr. Li has successfully applied such models in previous National Institutes of Health (NIH) funded clinical trial studies¹⁰¹⁻¹⁰³. Although our past observational and randomized controlled studies showed low prevalence of missing host and clinical data in this population, we plan to use multiple imputation models to handle missing data as necessary.

While DTG has largely replaced EFV for first-line ART in the catchment population, some METHOD participants may be treated with EFV-containing regimens. These individuals would be expected to have lower metformin exposure than participants on DTG-containing regimens, which might influence HDT efficacy or risk of AEs. The statistical analysis will consider stratification by EFV levels in the descriptive and covariate-adjusted analyses. We will carefully test interactions between the treatment and EFV, if statistically significant interaction is detected, we will stratify the analysis by EFV. Otherwise, we will adjust EFV as a covariate in the analysis.

14.4 Sample Size Considerations

The planned systems immunology analyses for the omics control group do not lend themselves to typical sample size estimation used for clinical studies with limited variables. Instead, the sample size per group (N=30) is justified by results from previously published proteomic, transcriptomic and/or flow cytometric studies involving TB that identified significant differences in protein and mRNA levels, leukocyte subsets, and gene expression pathways with sample sizes of 30 or less^{33,139}. Having an equal sample for all three groups (control, ATT alone, ATT plus metformin maximizes the power and discovery potential of the study. Unbalanced group sizes and reduce statistical power as cause difficulties in machine learning especially with very skewed numbers.

The primary objective for the treatment arms is to determine tolerability and safety of metformin added to standard ATT and ART in the treatment of TB/HIV co-infection. The most likely potential barrier to widespread adoption of metformin for this indication is GI toxicity. Although GI AEs are relatively common during standard TB treatment, severe or serious GI AEs are distinctly uncommon. In a TB-HDT study funded by the Bill and Melinda Gates Foundation and currently underway at Tembisa, participants are randomly assigned to receive 1 of 4 adjunctive HDTs plus rifabutin-substituted TB treatment, or that treatment alone. In an interim analysis, 38 instances of non-hepatic GI AEs were noted among 120 enrolled participants. Only 2 were serious and/or severe, requiring temporary or permanent discontinuation of treatment. Of these, one was attributed to the study drug. From this, we estimate that the expected rate of non-hepatic GI AEs leading to temporary or permanent discontinuation of standard treatment is 1-2%, i.e., 98-99% of patients are able to continue treatment without such events. The following table indicates sample size requirements for a range of event frequencies and non-inferiority margins, assuming $\alpha=5\%$, $\beta=80\%$, and one-side non-inferiority test for proportions is used.

Table 4. Primary endpoint sample size requirements

Success rate in controls	Success rate in experimental arm	Non-inferiority margins	Minimum sample size per arm
--------------------------	----------------------------------	-------------------------	-----------------------------

99	99	5	49
99	99	6	35
99	99	7	25
99	99	8	20
98	98	5	97
98	98	6	68
98	98	7	50
98	98	8	38

Regarding statistical analysis for the PK/PD study, data from intensive sampling (N=10 for metformin, or N=20 for the TB drugs, 8 samples each), plus sparse sampling (N=46 for metformin and N=92 for TB drugs, 3 samples each) are sufficient to support the determination of the structural model and characterization of the typical population parameters for the drugs in the study. A 1-compartment model is sufficient to describe the PK of metformin, rifampicin, and pyrazinamide. A 2-compartment disposition model is suitable for isoniazid, and therefore we will need to use Bayesian priors from our previously proposed models. The size of the sparsely sampled cohort will provide a reasonably diverse range of covariate values for the model and will enable identification of significant covariate effects. The amount of individual data from each sparsely sampled participant (3 samples) will provide reasonably accurate estimates of AUC and Cmax. The sample size for the PK/PD analysis of the first-line TB drug efficacy is larger than that used in previous studies that successfully identified PK/PD relationships for treatment ^{128;129}.

A total of 112 participants will enter this 2-arm parallel-design study in order to enroll 100 participants (50 per arm) fully evaluable for the secondary endpoint of sputum culture conversion. “Evaluable” is defined as having sufficient culture data to determine time to sputum culture conversion by week 12. Our main efficacy endpoint will compare the time to conversion in the metformin arm with the control arm. Based on the results of the PanACEA MAMS-TB trial ⁵⁸, we anticipate a median of 62 days (2 months) to stable culture conversion in the treatment control group (ATT without metformin). The probability is 80 percent that the study will detect a treatment difference at a two sided 5.0 percent significance level if the true hazard ratio is 1.94 ¹³⁰. This HR is somewhat higher than that detected in the high dose rifampicin arm of the PanACEA trial. These estimates are based on the assumptions that the accrual period will be 120 weeks, the follow up period for the endpoint will be 3 months, and the median time to culture conversion is 2 months in the control arm.

The proportion of participants with cultures having growth of Mtb on solid medium after 8 weeks of treatment is an alternative microbiological endpoint ⁹⁸. It has the benefit of validation for the prediction of recurrence rates in TB treatment-shortening trials ^{131;132}. Assumptions for statistical power analysis were based on results of four recent Phase 3 trials reporting the proportions with positive cultures at 2 months using solid media in the control (HREZ) arms as shown in Table 6. We estimated the detectable treatment effects (reduction in percentages) for a study with 100 total evaluable participants with complete information to detect difference between the standard treatment (control) and metformin arms, over a range of proportions in the two arms. Using one-sided Fisher’s exact test for testing two independent proportions at 5% significance level with at least 80% power, the trial can detect a reduction in proportions of 14.3%, 14.8%, 15.3% and 15.8% when the proportions of the treatment control group are 15%, 16%, 17% and 18%, respectively. Given that this study is a Phase 2A trial, it is not powered to detect a modest to weak metformin treatment effect. However, the study, with N=50 per arm, can provide a reasonable precision when estimating sputum culture positive proportions. When the true proportions are 2.5%, 5%, 7.5%, 10%, 12.5% and 15%, the precision of the estimate (half width of 95% CI) can

achieve 5.7%, 7.2%, 8.3%, 9.3%, 10.1% and 10.7%, respectively. These estimates will be informative to the design of the subsequent, larger Phase 3 trial.

Table 5. Sample size estimation for time to conversion

			Metform vs control
N per arm (evaluable)	α	β	HR
80	.05	.8	1.70
60	.05	.8	1.84
50	.05	.8	1.94
40	.05	.8	2.08

Table 6. Expected sputum culture-positive proportions in treatment control participants

Study	N	Metform vs control
Benator (2002) ¹⁰⁹	443	0.176
REMox (2014) ⁴	502	0.164
OFLOTUB (2014) ⁵	676	0.167
RIFAQUIN (2014) ²	160	0.076

14.5 Enrollment/Stratification/Randomization/Blinding Procedures/Unblinding Procedures

Candidates for participation in the METHOD trial will be identified and recruited from Primary Health Care clinics City of Ekurhuleni and City of Tshwane Districts and Primary Health Care clinics in the City of Johannesburg as well as and Nelson Mandela Bay area, Sub district A and C within the Province of Eastern Cape, South Africa. Participants will be identified using the TB Registry. Outstanding Xpert/RIF results will be followed using the NHLS TrakCare web portal. HIV status and CD4+ T cell count will be known for some but not all participants prior to screening. The HIV results will be accessed using the above system for candidates with previously known HIV status. The primary care clinic staff or (with permission from the District Department of Health) will introduce/refer selected candidates (with their permission) to the Aurum Tembisa CRC and Isango Lethemba TB Research Unit study team, allowing the team to look up additional laboratory results and initiate screening and enrollment (Appendix A). Recruiting will also be implemented through The Aurum Institute HIV Counselling and Testing program operating in the Tembisa catchment areas.

A cohort of 112 participants is planned (56 per arm), aiming to retain 100 participants with evaluable data for analysis (14 buffer based on retention in nine completed or active trials at Tembisa and Isango Lethemba TB Research Unit). We will enroll TB/HIV⁺ individuals (N=30) to provide baseline values for proteomic, transcriptomic and flow cytometry studies. The TB and HIV status of the control participants will be confirmed by chest x-ray, sputum culture and HIV serotesting. Thus, maximum total enrollment including both interventional study arms and omics control group will be 142 individuals. Previous studies at The Aurum Tembisa CRC have had excellent retention rates thanks to the Tembisa CRC Community Engagement Unit. Details of retention are presented in Protocol Section 9.3.

A stratified recruitment and randomization approach with pre-determined quota is deemed unfeasible owing to the small sample size and skewing of relevant variables in the population being studied. As an example, radiographic TB severity is generally lower in HIV-positive individuals than in the general population. However, post hoc stratified analysis will assess

potential effect modifications (or heterogeneity in treatment effects) of host factors including age, sex, and radiographic TB severity. Although the current trial is not powered to detect such variations in treatment effects, such data will be informative to the design of the subsequent larger Phase III trial and help us determine if potential heterogeneity in treatment effects should be factored into the trial design. Briefly, in the statistical analysis, participants will be stratified into 8 strata according to sex (male vs female), age (>45 vs <45 y) and TB severity (minimal/moderate vs advanced) as determined by the radiographic extent of disease at enrollment. Since radiographic severity reflects the interaction of host immune status and *Mtb*, and since it is correlated with CD4 counts, effect modification by chest x-ray is possible and worth evaluating. Participants will be randomized by block sizes of 2 and 4 in random order with equal probability for either arm. The statistician will create a randomization program based on the “ralloc” procedure available in Stata MP 15 (Stata Corp., College Station, TX). Seed number for the randomization program will be preserved so the randomization process can be tracked and audited as necessary. A non-informative coding for the two arms (i.e., A vs B) will be used to blind the treatment status during the trial. Randomization sequences by stratum generated a priori will be done manually via opaque, sealed envelopes, assigned sequentially by the Pharmacist once the Investigators have confirmed eligibility of the participant to be randomized. Only once the envelope has been assigned will the assignment be known to the study team, as this is an open-label trial. A specific quota for each stratum is no longer necessary and thus will not be implemented. Treatment randomizations are blinded to the investigators and staff. Treatment status unblinding will be allowed only if needed for participant safety or as permitted jointly by the investigators, the DSMB and the sponsor.

Samples from the omics control group and the interventional arm participants in the METHOD trial will be prepared at Tembisa and delivered to the Singhal laboratory in a de-identified manner. When the interventional study is unblinded, Dr. Singhal will receive information for group assignment that will not contain personal identifying information.

14.6 Participant Enrollment and Follow-up

The METHOD trial will rely on two sites, the Aurum Tembisa CRC and Isango Lethemba TB Research Unit. Based on the sample size estimation (Section 14.4.), the goal is to follow 100 TB/HIV co-infected participants study completion. We anticipate enrolling an additional 12 participants to make up for enrolled participants who may be lost to follow-up or withdrawn from participation. It is further anticipated that to enroll the combined total of 112 participants, we will need to screen twice that number or 224 candidates. This estimate is based on the typical ratio of screened to enrolled participants in other clinical trials at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit on the typical retention rates in those studies. Participants in the omics control group finish their involvement with the study at the end of the enrollment visit.

15 Data and Safety Monitoring

A Data Safety and Monitoring Board (DSMB) for the METHOD trial will be established and supported by the Aurum Institute, with membership reviewed and approved by DAIDS. The DSMB will operate as detailed in the document “Charter for the Data and Safety Monitoring Boards of the Division of AIDS”, version 5.0 (06/24/2015). The DSMB will meet semiannually to assess safety and efficacy data on each arm of the study. The DSMB will review aggregate safety data for increased rate of occurrence of serious suspected adverse reactions. The METHOD trial Chief Statistician (Dr. Wenjun Li) will provide the appropriate reports for DSMB review.

The implementation of the METHOD Trial will be overseen by Drs. Wallis, Kornfeld, Churchyard, and the site PIs from Tembisa site and Isango Lethemba TB Research Unit. Members of this oversight group, along with other members of the site teams as needed, will meet bi-weekly or more frequently if needed. The purpose of these team meetings is to monitor the implementation and progress of the trial, review information from external sources relevant to the design and conduct of the study, discuss recommendations from the DSMB, and address any barriers to productivity or other matters arising. Drs. Wallis, Kornfeld, Churchyard, and the site PIs from Tembisa and Isango Lethemba TB Research Unit will conduct an investigators’ meeting at least quarterly to discuss any issues or events affecting participant safety and performance of the site staff.

An Independent Safety Monitor (ISM) will be appointed to provide unbiased oversight of operations at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit. The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. This ISM is identified by the investigator site and approved by DAIDS. The ISM will review SAEs and other AEs as needed and provide an independent assessment to DAIDS. The study site will have an ISM with experience in infectious diseases and/or internal medicine.

15.1 Planned Interim Analyses and Stopping Guidelines

Analyses and Stopping guidelines are applicable to the intervention group, not the omics control group.

Safety and data integrity will be regularly monitored and reported to the DSMB, investigation team and DAIDS designated officers. Quarterly safety and data monitoring reports will be provided. Severe adverse events will be communicated with the DSMB and investigation team immediately. Interim safety review will be conducted when week-8 results are available from the first 50% of participants enrolled. This analysis will focus on safety measures, such as proportions of participants who are unable to continue study drug due to gastrointestinal symptoms and/or other important side effects/severe adverse events. The information will be shared with the DSMB to determine if the continuation, modification, or termination of the trial after the early phase is justified. Efficacy data will also be evaluated in the first interim analysis to place the safety data in context, recognizing that the sample size is too small to power interim efficacy analysis.

The information will be used to determine whether the trial can achieve the analytic goal given the observed variances in treatment responses (e.g., if observed variations are substantially larger than expected, the trial lacks adequate statistical power as planned, and the logistics do not allow drastic increase in trial size, an early stop may be justified). The interim analysis results will be shared with NIAID, DSMB and investigation team, for data-driven joint decisions. Exact

stopping rules will be developed jointly with NIAID, DSMB and the investigation team before or when the trial begins.

15.1.1 Interim Safety Review

Interim safety review is applicable to the intervention group, not the omics control group. Subsequent review of serious, unexpected, and related AEs by the DSMB, ISM, EC/IRB, SAHPRA, or other relevant local regulatory authorities may result in suspension of further trial interventions. If more than 20% of the first 20 participants in the metformin arm permanently discontinue treatment for any reason before completing the planned treatment, a DSMB review will be triggered to determine if enrollment should be terminated. If fewer than 20% of the first 20 participants in the metformin arm permanently discontinue treatment for any reason and study enrollment continues, then the threshold for triggering DSMB review will be reduced to 10% of the subsequently enrolled participants.

Among potential SAE/AEs associated with metformin, the following will be carefully scrutinized:

1. Gastrointestinal intolerance
2. Weight loss >6% of baseline
3. Hypoglycemia
4. Lactic acidosis

These parameters are assessed at every study visit and capture in the follow-up visit CRFs and in AE reporting CRFs.

Based on the available data, the DSMB will advise DAIDS of its recommendations after each meeting. Examples of findings that might trigger a safety review are the number of SAEs overall or in particular subgroup, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events.

15.1.2 Interim Efficacy Review

No interim efficacy review is planned.

15.2 Analysis Plan

The analysis plan is applicable to the intervention group, not the omics control group.

Primary safety and efficacy endpoints will be evaluated based on a modified intention-to-treat population, consisting of all participants who underwent randomization and received the study medication at least once, but excluding participants whose initial laboratory evaluation subsequently indicated study ineligibility due to negative baseline Mtb culture or positive baseline culture with resistance to isoniazid, rifampicin, or pyrazinamide. The modified intention-to-treat analysis will include all participants who have taken at least one dose of the study medications during the continuation phase of ATT. This group will be treated as the any-exposure population. In addition, participants who took at least 80% of all doses over a period of 3 months will be included in a per-protocol population. A secondary analysis will examine efficacy endpoints in the any-exposure vs per-protocol population who adhered perfectly to the intervention and trial instructions. In view of the potential for discordant results between solid and MGIT cultures, participants with negative solid cultures at baseline or with contaminated solid cultures at week 8 (month 2) will be excluded from the week 8 (month 2) analysis; however, they will still be included

in analysis of MGIT results if baseline MGIT will be positive, and in analysis of the secondary outcomes.

Briefly, the following general statistical approaches will be used to evaluate the primary safety and efficacy endpoints. For safety endpoints and tolerability of metformin, average and median numbers of grade 3, 4 and 5 adverse events will be tabulated by treatment arm, with and without stratification by sex, age, and TB severity whenever sample sizes are adequate. Since the number of adverse events are likely highly skewed, Poisson or negative binomial distribution-based test will be used to evaluate whether number of adverse events differs between arms (crude analysis). Poisson regression models will be used to compare rates of adverse events between the two arms, with and without stratification by sex, age, and TB severity. Adjustment for other unbalanced patient factors will be considered as needed. In case the adverse events are rare or existence of over-dispersion, negative binomial models will be used. Using these models, we also will attempt to identify host factors predictive of potential adverse events. In addition, similar analyses will be conducted for each type and grade of adverse events (e.g., intolerance). If adverse events are rare and typical statistical models cannot be used effectively for small numbers, we will enumerate the events, overall and by sex, age group and severity.

For the microbiological efficacy endpoint of time to sputum liquid culture conversion, data will be analyzed and compared between the metformin and standard treatment arms using log-rank test (crude analysis) and Cox proportional hazards models with adjustment for relevant unbalanced covariates. In addition, heterogeneity in metformin treatment effects on time will be evaluated by stratifying the analysis by sex (male vs female), age group (<45 vs ≥45y) and TB severity (minimal/moderate vs severe). Further, multivariable Cox regression models will be used to examine differential treatment effects by these variables, or certain combinations of any of the three factors (interactions), adjusting for other relevant host factors (controlling for confounding). Interaction terms between treatment indicators (or metformin dosage variable) and host factors (sex, age and severity) will be included in the models when stratum sample sizes support. Statistically significant interactions may indicate potential heterogeneous MET effects by these host factors, and the need for in-depth analysis and reporting by subgroups. Such analyses will help answer clinical questions such as whether metformin effects will be stronger among or limited to certain patient subgroups. The possibility of participant mortality during the time to conversion window will be addressed using two approaches. The first is to consider death as a negative conversion outcome at the time of death; this may apply to patients whose cause of death is adjudicated to be due to TB. The second is to censor the patients who died of non-TB related cause(s), at the time of death.

For the complementary microbiological efficacy endpoint of solid culture status at month-2, the proportions of positive cultures after 8 weeks of ATT will be tabulated and compared between the metformin and standard treatment arms using Chi2 test (crude analysis), overall and stratified by sex, age group (<45 vs ≥45y) and TB severity (mild vs moderate vs severe). The purpose of the stratified analysis is to evaluate potential heterogeneity in metformin treatment effects by these host factors when stratum sample sizes support. Further, multi-variable logistic regression model will be used to estimate treatment effects with adjustment for relevant unbalanced baseline covariates (e.g., medications and comorbidities), overall and by sex, age group and severity. Selection of covariates will be guided by clinicians and literature, and we will use as backwards stepwise variable election method. In the models, we will evaluate the interaction terms between treatment indicator (and dosage variable) and these host factors. Statistically significant interactions indicate possible heterogeneity in metformin effects by one or more or combinations of these host factors, and the need to analyze and report trial outcomes stratified by these host factors. We will thus conduct and report in-depth subgroup analysis. Such analyses will help

answer clinical questions such as whether metformin effects will be stronger among or limited to certain patient subgroups. We are fully aware that this small sized trial is not powered to detect modest to small subgroup differences. However, such analyses can be informative to the design of the subsequent larger Phase 3 trial and is thus highly desirable.

To determine whether metformin added to standard ATT in participants co-infected with and treated for HIV improves respiratory health after TB treatment completion, we will analyze between-arm differences in several secondary endpoints of change in radiographic score and SpO₂, 6MWT distance-saturation product, spirometric values and SGRQ score. Briefly, radiographic score and SpO₂, 6MWT distance-saturation product, spirometric values and SGRQ score will be tabulated by arm and time point, with and without stratification by sex, age and TB severity when stratum sample sizes allow. Pre-post changes in these outcomes will also be tabulated in a similar fashion. Wilcoxon rank sum tests will be used to evaluate whether pre-post changes in these respiratory outcomes differ between two arms (crude comparison). Further, we will use general or generalized linear mixed models to obtain covariate-adjusted metformin treatment effects on these respiratory outcomes. Each of these respiratory outcomes will be analyzed using a separate model. Data on all time-points will be used for modeling. Serial correlations between measurements on the same participant will be modeled with a proper variance structure (e.g., first order autoregressiveness or unstructured covariance). The covariate-adjusted models will include sex, age and TB severity as well as other potential predictive patient factors. Potential effect modifications among these predictors will be explored. The metformin treatment effects can be determined by the coefficients of the interaction terms between metformin treatment indicator and post-treatment time point indicators. If significant interaction terms between metformin indicator and other host factors (e.g., sex, age group and TB severity) are observed, we will conduct in-depth subgroup analysis, and report results stratified by these variables.

To determine whether metformin added to standard ATT in participants co-infected with and treated for HIV improves HIV outcomes, we will evaluate between-arm differences in pre-post changes in the exploratory endpoints of viral load and CD4+ T cell count. As viral load and CD4+ T cell counts usually have skewed distribution, we will consider logarithmic transformation and use of quantile regression models. Quantile regression models will allow us to evaluate the metformin effect on changes in distributional characteristics of viral load and CD4 counts (e.g., significant decreases in lower quartile or median values of viral loads and increases in median and upper quartile values of CD4+ counts when treated with metformin). Statistical comparisons are very similar to those for Study Objective 3, except the outcomes and the underlying statistical models (and thus not repeated).

Finally, to determine whether metformin added to standard ATT in participants co-infected with and treated for HIV improves TB treatment outcomes, we will evaluate between-arm differences in the secondary endpoints of cure, failure, death, default and relapse. The proportions of cure, treatment failure, death, default, and relapse will be tabulated and compared between the two arms using Chi² test (crude comparison) and multivariable logistic regression models adjusting for relevant unbalanced covariates. For rare events or groups with small numbers, such as death, we will also consider Fisher's exact test and/or exact logistic regression models. In addition, heterogeneity in these outcomes will be compared between the two arms, stratified by sex, age group and TB severity, with and without covariate adjustment. As time of death or relapse will be recorded, we will also consider use of time-to-event models such as Weibull or Cox proportional hazard regression models.

In all these analyses, model performance, distributional assumptions and functional forms will be carefully examined. To address issues regarding participant dropout, we will compare characteristics of the completers and the dropouts. If dropout is prevalent (e.g., >10%), we will build a logistic model to examine whether the dropouts are related to treatment arm and host factors.

We will use the model to estimate the probability of dropout and use the probability for inverse probability weighting in the final analysis (marginal structural equation models). The bootstrapping method will be used to account for variances in estimated probabilities of dropout. The METHOD trial Chief Statistician, Dr. Wenjun Li, has successfully applied similar models in previous NIH-funded clinical trial studies. Although our past observational and randomized controlled studies showed low prevalence of missing host and clinical data in this population, we plan to use multiple imputation models to handle missing data as necessary.

16 DATA HANDLING AND RECORDKEEPING

The Data Management Department of the Aurum Institute will provide data management services for this project. Data collection will be managed either by paper CRFs or electronic data capture (EDC) or a combination of those methods. Upon entry into the system, full audit trails will be maintained for all study data. Data is housed in a secure database with limited username and password access. Electronic data is backed up at standardized intervals and kept in a secure location. Datasets are managed in STATA and posted to Aurum's secure FTP site for access by authorized study personnel. Ongoing data monitoring is provided for source document verification for critical variables. Paper documentation is housed in Aurum's archives for three years before movement to permanent storage facilities for the required timeframe. Final, clean datasets will be made available to stakeholders within 180 days of the last participant's last visit. Prior to that time, access to data must be requested from the Aurum Institute Data Management Center and may require the approval of the study investigator team.

AURUM has selected Merge Zelta as a database platform for this study, and to that end has four certified database designers within the Data Management department. Zelta is a cloud-based system that allows for EDC, capture from paper CRFs, or direct data uploads. It is flexible and easy to use for both data entry staff and site staff in the field using it to track participant data and status and it is 21 CFR Part 11 compliant. It also has the ability to upload DICOM images for study team review. From data entry screens, to validation, to querying and reporting, Zelta has proven to be faster and easier to use than other database systems Aurum has used or implemented in the past.

Data collected on CRFs is identified by a unique study identifier and all study information is kept separately from a participant's personal identifying information. All communications regarding a participant use his/her study identifier. Access to the Zelta database is by username and password only and is managed by the Data Management team. Participant paper files are stored in secure, locked file cabinets while on site and are stored in the secured Aurum Archives once delivered to the central data management office. Documents are stored on-site for 3 years before being moved to a secure facility for the duration of the required storage time.

CSV datasets of all CRFs are generated on a weekly basis and read into STATA for data management and analysis. These datasets and merged datasets will be used for interim analyses as needed. A database design document can be created at any time (scheduled time points for generation for review and sign-off are: pre-go-live, go-live and study close out) that lists all study processes, user access, tables, variables, code lists, queries, and reports. This document compiles both the data dictionary and the codebooks.

16.1 Data Management Responsibilities

Data management for the METHOD trial will follow data established practices specified in the Aurum Institute SOPs. The Data Management Plan will itemize all deliverables and timelines for data management for this study. The Data Management team is responsible for providing the study database and CRFs (paper and electronic) as well as all instructions and documentation to support the study database and training resources for the site. Data Management staff provide initial and follow-up training for study staff as required and ensure quality assurance and quality control of the data management system. Real time verification checks are provided upon data entry to ensure completeness (no missing data), accuracy (range and cross-form checks), and reliability. In addition, standard and ad hoc reports are available to the study team upon request.

Data Management also provides source document verification between source documents/CRFs and the database for critical variables as defined by the study team.

The protocol Chair and Co-Chair (Drs. Wallis and Kornfeld, respectively) have the primary responsibility for interpretation of clinical data. Dr. Singhal and Prof. Maartens have the primary responsibility for interpretation of transcriptomic, proteomic, flow cytometry and PK data generated at their institutions. The Chief Statistician (Dr. Li) has primary responsibility for the statistical analysis of all METHOD trial data.

16.2 Essential/Source Documents and Access to Source Data/Documents

Source documents are defined as all information in original records and copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents will include, but are not limited to, progress notes, electronic data, screening logs, and recorded data from automated instruments. All source documents and source data are considered essential documents.

All essential documents pertaining to this trial will be maintained within data management facilities that ensure privacy, confidentiality, security, and accessibility when the clinical research is being conducted and after the research is completed at the clinical research site. Responsibility for the delegation of access of such data to site staff will be overseen by the investigators at the Tembisa CRC and Isango Lethemba TB Research Unit. The investigator will in accordance with ICH-GCP guidelines permit trial-related monitoring, audits, Independent Ethics Committee/Institutional Review Board (IEC/IRB) review and regulatory inspections, providing such authorized persons direct access to all essential documents. All records will be archived in a secure storage facility for at least ten years after the completion of the study. The relevant SOPs are available.

16.3 Quality Control and Quality Assurance

Clinical Quality Management Plan (CQMP)

The METHOD trial will follow procedures established at the study sites to ensure the quality of clinical trial data as detailed in their site/study Quality Management Plans

Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and the compliance and quality assurance groups of all participating institutions with regard to study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

17 CLINICAL SITE MONITORING

A Contract Research Organization (CRO) will provide project management and monitoring and will be responsible for ensuring all procedures are conducted in accordance with the protocol and all applicable regulations.

Site monitors from the CRO will visit participating clinical research sites to review participants records, including consent forms, CRFs, medical records (e.g., physicians' progress notes, nurses' notes, individuals' hospital charts), and laboratory records to ensure protection of study participants, compliance with the EC/IRB approved protocol/amendments, and accuracy and completeness of records. The monitors will inspect sites' regulatory files to ensure that local regulatory requirements, in addition to U.S. Federal regulations, are being followed. They will also inspect sites' pharmacies to review product storage and management. A separate monitoring plan document will be developed to describe who will conduct the monitoring, the detailed scope of the monitoring to be performed, and the frequency of monitoring visits.

17.1 Project Management

The CRO project manager (PM) will collaborate with Aurum to manage the conduct of the study. The PM tasks and activities include but are not limited to:

- Arranging, preparing, and conducting project team training, including self-training of all Clinical Research Associates.
- The customising of appropriate monitoring tools and set-up of electronic tracking systems for local project management.
- Aurum will be responsible for the set-up and maintenance of an in-house paper Trial Master File (TMF) for the sites,
- The oversight and tracking of study objectives, site issues, timelines and budget.
- Supervision of the study CRAs and internal review of site visit reports.
- 2 Co-monitoring visits at site where PM intervention is indicated (post Sponsor authorisation).

17.2 Clinical Monitoring and Site Management

This study will be monitored utilizing a risk-based monitoring model and monitoring activities will include:

- Review of site essential documents.
- Conduct of virtual Site Initiation Visits, Interim Monitoring Visits and Close-Out Visits. Prior Sponsor authorisation will be sought should additional on-site visits be deemed necessary, over and above the budget estimates.
 - Non-data activities i.e., IP management (confirmation of receipt & storage, 100% accountability for Metformin & coordination of return to IP vendor), regulatory file review.
 - 100% Informed Consent Form review for all enrolled subjects only
 - 100% Investigational Product accountability (Metformin Arm)
 - 100% SDV of five enrolled participants in treatment group. (3 at Tembisa CRS and 2 at CHRU Isango Lethemba TB CRS)
 - 25% SDV of remaining enrolled subjects in treatment arm (n=107).
 - 25% SDV of control group (n=30).
 - 100% SAE review: 11 SAEs expected.

- Collection and review of site essential documents for IP release and site readiness
- Regular inter-visit site contact and follow-up as appropriate.
- Site support activities including re-training

17.3 Systematic off-site document and data review and analysis

Before each visit, the CRA will contact the site to schedule a mutually convenient visit date. After a date has been set, a letter will be sent to the Investigator and other appropriate study staff to confirm the date and time and to explain objectives of the visit. After the visit, the CRA will send a follow-up letter to the investigator and other appropriate study staff, detailing accomplishments, action items, and any GCP non-compliances. The monitoring visits should be conducted in accordance with the Scope of Work compiled and agreed upon by both Aurum and the CRO.

17.4 On-Site Monitoring

As for most grant funded studies, an onsite QC monitor will monitor all source documents to ensure all procedures were conducted and will do source document verification of selected data captured of all enrolled participants at each site. On-Site monitoring should be conducted in accordance with the Site Monitoring Plan and completed within timelines defined in the plan. In summary, all data capture is to be completed, source verified and database queries raised and answered within 5 working days of the completed participant visit. AURUM and/or the CRO will track this metrics in real-time and address timeline associated issues on needs and or case-by-case basis.

17.5 Sponsor Audits

The purpose of these additional auditing visits is to validate data integrity, review additional participant records and to assess the performance of the CRO. A formal audit plan developed by Aurum, for this purpose will be shared prior to commencement of the screening and enrollment epoch.

18 HUMAN SUBJECTS PROTECTION

18.1 Ethics Committee/Institutional Review Board

Human subject research activities are subject to the U.S. Federal Policy for the Protection of Human Subjects (also known as the Common Rule). This clinical trial will be conducted according to the ethical principles of the World Medical Association's Declaration of Helsinki (updated October 2013), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practice (ICH-GCP), and all local regulatory requirements as applicable.

Applications have been approved by the EC/IRB of each participating institution and by SAHPRA, whose approval is required in order to conduct a clinical trial in South Africa. The purpose of the SAHPRA is to establish the safety, quality, and efficacy of the medication in keeping with the current knowledge at the time of consideration of data in the conduct of the clinical trial.

Collaborating institutions, University of Massachusetts Medical School, the Aurum Institute, SlgN, University of Cape Town, and NICD (Wits Health Consortium), have a current U.S. Federal Wide Assurance (FWA) issued by the U.S. Office for Human Research Protections (OHRP). UMMS will obtain signed assurances from each participating institution, their FWA numbers and copies of their IRB approvals.

Institutional Review Boards/Ethics Committee

University of Massachusetts Medical School

Dr. Hardy Kornfeld, the Protocol Co-Chair and Contact PI at UMMS, is responsible for overall project management, oversight of the clinical trial, fundamental research activities and communications, communication with NIAID, statistical analysis, and compliance with US regulations. The UMMS IRB signed an IRB Authorization Agreement with WHREC.

The Aurum Institute

Under the direction of Prof Robert Wallis (MPI, Investigator of Record and Protocol Chair), The Aurum Institute will operate the research site and implement the clinical protocol in compliance with local regulations.

Singapore Immunology Network

Under the direction of Dr. Amit Singhal (MPI), the Singapore Immunology Network (SlgN) will implement the basic science studies (Aim 2 in the grant). His group will perform systems biology studies on participant samples. As SlgN will receive de-identified samples and will not have access to personal identifying information, SlgN's Ethics Committee (National University of Singapore, IRB) considers this a low risk project, and will conduct an expedited review.

University of Cape Town

Prof. Gary Maartens, Co-Investigator at University of Cape Town (UCT), and his group will develop the metformin assay; perform drug assays for metformin, isoniazid, rifampicin and pyrazinamide; and analyze the pharmacokinetic data used non-linear mixed effects modelling. Once the WHREC approves the protocol, it will go through expedited review at the UCT Human Research Ethics Committee. Since UCT will have no access to personal identifying identifications, the EC considers it low risk and will conduct an expedited review.

Protection of Human Subjects Training and Certification

All personnel will complete an accredited educational program on the protection of human research participants and have current training certifications. All personnel at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit undergo GCP training upon hiring and will be recertified at least every three years. All personnel at UMMS complete the NIH certified online human subjects protection (HSP) training course.

Aurum Regulatory Manager, Ms. Stacey Lawrence will oversee study regulatory requirements. The PD/PIs at each of the collaborating institutions are responsible for ensuring that all personnel receive training appropriate for the individual's level of involvement in research. The PD/PIs are also responsible for ensuring that new staff members receive this training within 90 days and are adequately supervised during the period prior to training. Training records will be maintained at the site and include the trainee's name, date of training, name/affiliation of trainer, title of course, and primary contents covered in the training. Personnel will receive training prior to the screening or enrollment of the first participant and repeat this training every three years. The PD/PIs are responsible for managing the regulatory requirements at their respective institutions.

This clinical trial will be conducted at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit, under the auspices of The Aurum Institute and the WHREC. Ethical approaches to recruitment and principles and process for informed consent are adhered to. Standard systems are implemented to avoid breaches in confidentiality where all participants are assigned a unique identification number and confidential information is stored safely. Systems to monitor reported AEs and social harms are in place. Risk reduction counselling and referral for treatment are meticulously and consistently implemented.

Quality control systems are implemented to ensure that research procedures involving human are maintained on an ongoing basis according to established SOPs for the conduct of clinical trials at the CRC and according to protocol requirements. This study adheres to the local Good Clinical Practice Guidelines (GCP guidelines), ICH-GCP guidelines as well as NIH guidelines to ensure that all regulatory and ethical considerations have been met, that the rights and safety of the study participants have been maintained, and that all the protocol requirements are followed.

The DAIDS Medical Officer (MO)/Program Officer (PO) will provide a preliminary assessment of the accuracy and completeness of the informed consent document prior to the Scientific Review Committee (SRC). The DAIDS MO/PO provides oversight and review of IC documents submitted to DAIDS and for ensuring that the IRB/EC has reviewed and approved the ICF reflecting the current version of the protocol. The DAIDS Regulatory Affairs Branch (RAB) Human Subjects Protection (HSP) Team provides oversight and review of the submitted IC document (s) at the time of SRC review and, if applicable, full regulatory review.

Each institution (UMMS, The Aurum Institute, SIgN, and University of Cape Town) will ensure that this protocol, informed consent documents, and all study-related documents are reviewed and approved by their respective EC/IRB prior to protocol implementation. Written informed consent will be obtained from each participant prior to any protocol-specified procedures being conducted. The protocol, informed consent and assent forms will be reviewed and approved by the IRB or EC prior to any protocol-specified procedures being conducted.

The PD/PIs are responsible for ensuring that the protocol is reviewed by the EC/IRB of their respective institutions. The investigators will inform the IRB/EC as to the progress of the study at applicable as defined by the IRB/EC policy.

Protocol Amendments

The study team will send all protocol amendments and consent changes to the DAIDS Medical Officer for review prior to submitting the protocol amendments and consent changes to the involved EC/IRBs.

Letters of Amendment

All DAIDS-approved changes to the protocol, informed consent(s) and other study-related documents. Amendments and Letters of Amendment (LoA) will be approved by sites' EC/IRB prior to implementation. This protocol includes the applicable DAIDS-approved template protocol registration language for amendments and registrations. See section 19.1 for protocol registration and LoA registration. If enrolled participants are in apparent immediate danger, the IoR or designee may immediately implement changes to the research to eliminate the danger before EC/IRB approval is obtained. These instances will be documented in writing in the site's regulatory file and EC/IRB approval will be obtained at the earliest feasible date.

Continuing Review and Approval

EC/IRB continuing review and approval will be obtained at least once per year. If there is a lapse in continuing review, all ongoing research activities will stop, unless the investigator determines that it is in the best interest of already-enrolled participants to continue their study-related activities. However, new participants will not be enrolled on the study until EC/IRB approval to continue the research is obtained.

18.2 Vulnerable Participants

The METHOD trial will recruit and enroll vulnerable participants as defined under South African GCP: HIV+; unemployed; illiterate; females.

18.2.1 Pregnant women and fetuses

The METHOD trial will enroll adult participants only and pregnancy is an exclusion criterion for enrollment. Female participants who become pregnant during the study will be withdrawn (see Section 13.3).

18.2.2 Prisoners

No prisoners will be enrolled in the METHOD trial. Participants who become prisoners during the course of the study will be withdrawn.

18.2.3 Children

The METHOD trial will test the safety of metformin as HDT for TB in adults with HIV co-infection. Children will not participate in this trial on the basis of safety considerations and the evaluations necessary for the primary and secondary outcomes measures. While metformin appears to be safe in obese pediatric patients with type 2 DM ¹³⁴ nothing is known about its safety in non-diabetic children with HIV and TB co-infection. Furthermore, sputum culture has low sensitivity for TB diagnosis in children ¹³⁵ and this parameter is the basis for an important efficacy outcome measure of the study.

18.2.4 Illiterate participants

Participants who speak and understand the language used during the consent discussion and in the informed consent form (ICF) but who are unable to read or write (illiterate) can be enrolled in the METHOD trial by "making a mark" on the informed consent form as described in SOP "Obtaining Informed Consent" and the SOP "Consenting of Illiterate Participants". Briefly, consent must be obtained in the presence of an impartial witness who is independent of the study and who is present throughout the entire consent process. If the participant cannot understand any language into which the informed consent forms (ICFs) have been translated, consent may be obtained in the participant's preferred language in the presence of an impartial witness and a translator. The researcher must record on the ICF that consent was obtained verbally from a translator. The translator and the witness must complete and sign their respective sections of the ICF.

18.3 Informed Consent

18.3.1 Informed Consent Process

Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided by study staff to potential participants (and, with permission from potential participants, their families). Potential participants will receive counselling about study objectives and procedures, potential toxicities, and the informed consent process. Consent forms describing in detail the study interventions and products, study procedures and risks will be given to the participant and written documentation of informed consent will be required prior to starting intervention/administering study product. Consent forms will be IRB approved, will contain all the elements required by the ICH E6 Guideline for Good Clinical Practice and any additional elements required by local regulations. Forms written in English will be available for IRB and sponsor review. The approved forms will be translated into the Sepedi, Zulu, Xitsonga, Afrikaans, Xhosa and Sesotho languages.

The process of obtaining informed consent from literate and illiterate participants is described in Aurum's SOP "Obtaining Informed Consent", and the SOP "Consenting of Illiterate Participants" as noted in Section 18.2.4. The participant will sign or mark the informed consent document prior to any procedures being done specifically for the study. The participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed and dated informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing, to participants, that the quality of their medical care will not be adversely affected if they decline to participate in this study. During the informed consent process, the participant will receive information about compensation to reimburse them for their time expenses. This compensation does not represent payment for study participation. Participants will also be offered food in the form of snacks during most clinic visits and meals during the longer visit for PK sampling (V05).

18.3.2 Documentation of Informed Consent

Written informed consent will be obtained from all participants, including illiterate participants as described in Sections 18.2.4 and 18.3.1.

18.3.3 Waiver of Informed Consent

Waiver of informed consent by METHOD trial participants is not permitted.

18.3.4 Waiver of Documentation of Informed Consent

Waiver of informed consent by METHOD trial participants is not permitted.

18.3.5 Stored Samples and Associated Data Considerations

Samples of PBMC, plasma, serum, RNA and DNA will be isolated from blood samples collected at the Aurum Tembisa CRC only and stored on site at the Aurum Tembisa CRC. Samples from a subset of participants will be processed and used for flow cytometry, proteomics or transcriptomic evaluations within days to weeks, depending on the nature of the sample and batch wise considerations. Other samples may be stored for up to 5 years for future cytometry, proteomic and transcriptomic studies as determined by results from the initially evaluated samples and by available funding. Genomic studies related to the objectives of the METHOD trial will be limited to the epigenetic studies and pharmacogenomics analysis (single nucleotide polymorphisms) of genes linked to metformin elimination (see Section 10.1.8).

18.4 Risks

Risks for the omics control group are limited to minor risks associated with venipuncture (faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture), the inconvenience of multiple visits to the Aurum Tembisa CRC and Isango Lethemba TB Research Unit, and the risk for violation of privacy. The Aurum Tembisa CRC and Isango Lethemba TB Research Unit staff have extensive experience with phlebotomy in the context of clinical trials and they will ensure best practices to minimize the risk of these complications.

Treatment of TB and HIV co-infection is complex and subject to drug-drug interactions, adverse drug reactions and treatment failure. All treatment arm participants in the METHOD trial will meet the criteria for concurrent ATT and ART, and they would receive this treatment regardless of participation in the study. The risks shared by participants in the metformin and standard treatment arms of the trial are therefore limited to minor risks associated with venipuncture (faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture), the inconvenience of multiple visits to the Aurum Tembisa CRC and Isango Lethemba TB Research Unit and the risk for violation of privacy. Participants randomized to the metformin arm will have additional significant risks relating to adverse drug reactions specific to metformin and drug-drug interactions between metformin and ATT and ART drugs. Information on metformin-associated risks is presented in the package insert (Appendix O) and in Protocol Section 12. Key risks will be summarized here.

Lactic acidosis (LA) is a potentially life-threatening adverse effect that may be associated with metformin, particularly in the context of associated risk factors such as hypoperfusion or concurrent administration of drugs having mitochondrial toxicity. Previous generations of ART drugs had relatively high potential for mitochondrial toxicity. This risk is lower with newer generation agents but could occur with current first line ART in combination with metformin as suggested by rare case reports^{116;117}. Symptomatic hyperlactatemia without acidosis can also occur with metformin use. Lactate will be tested as needed if a participant reports compatible symptoms and metformin will be stopped if the lactate is >4.4 mmol/L. The development of LA is unpredictable making scheduled laboratory evaluations for prevention. Risk is mitigated by

excluding individuals with conditions thought to be risk factors for LA. If life-threatening LA is detected in a METHOD cohort participant, dialytic therapy is available close to the sites.

It is likely that high proportion of participants will experience mild, transient GI symptoms in the period of metformin initiation. In some individuals, GI intolerance is more severe and precludes continued use of the drug. Weight loss can result from anorexia and/or diarrhea associated with use of metformin. Baseline BMI $<17.0 \text{ kg/m}^2$ is an exclusion criterion. Body weight will be monitored at all follow-up visits and metformin stopped if and weight declines by $>6\%$ of the baseline value.

Participants in the metformin arm will be at risk for hypoglycemia although this rarely occurs without concurrent use of other anti-diabetic agents. Risk is mitigated by excluding individuals with DM, by giving metformin at a low total daily dose of 1000mg with meals, by counselling participants with respect to symptoms of hypoglycemia and actions to take if symptoms occur, and by checking random blood glucose levels with a glucometer at all scheduled visits during the period of study drug exposure (weeks 2 through 12). Use of metformin can be associated with vitamin B12 insufficiency, which will be mitigated by vitamin B12 supplementation. Other adverse effects that have been reported on metformin include hepatic dysfunction, flu-like symptoms and hypersensitivity.

18.5 Social Impact Events

Individuals enrolled in this study may experience personal problems resulting from the study participation. Such problems are termed social impact events. Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that participants may experience stigmatization or discrimination as a result of being perceived as being HIV-infected or having active TB disease. For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities. Problems may also occur in circumstances in which study participation is not disclosed, such as impact on employment related to time taken for study visits.

In the event that a participant reports a social impact event, every effort will be made by study staff to provide appropriate assistance, and/or referrals to appropriate resources. Social impact events are documented and reviewed on a scheduled basis by the protocol team leadership with the goal of reducing their incidence and enhancing the ability of study staff to mitigate them when possible.

Social impact events that are judged by the IoR/designee to be serious, unexpected, or more severe or frequent than anticipated, will be reported to the responsible site's EC/IRB promptly, or otherwise in accordance with the EC/IRB's requirements.

18.6 Benefits

Participants in the treatment arms of this study will benefit from careful medical care; and may benefit in the future from information learned in the study. They will also benefit from intensive monitoring of TB treatment that exceeds the standard of care in the community. Participants in the metformin arm could benefit from accelerated resolution of TB and relative preservation of lung function after ATT completion. If metformin demonstrates HDT efficacy in this study it could

lead to improved TB treatment in HIV+ individuals, which would be a considerable benefit to society. Participants in the omics control group will receive no benefit from their participation.

18.7 Compensation

Enrolled METHOD trial participants will be reimbursed R300 per standard visit for their time, inconvenience, and expenses. In the treatment arm of the study, participants will be reimbursed R450 at V06 (week 5) for sparse PK sampling and WBA that may take up to 6 hours. Participants sequentially selected for intensive PK sampling will be reimbursed R1000 per night at V06 (week 5) as this will be an overnight stay at the Aurum Pretoria CRC (this is only applicable to the Tembisa CRC).

18.8 Participant Privacy and Confidentiality

All participant-related information including case report forms, laboratory specimens, evaluation forms, reports, etc., will be kept strictly confidential. All records will be kept in a secure, double locked location and only research staff will have access to the records. Participants will be identified only by means of a coded number specific to each participant. All computerized databases will identify participants by numeric codes only and will be password-protected. Upon request, participant records will be made available to the study sponsor, the sponsor's monitoring representative, a participating pharmaceutical sponsor and representatives of applicable regulatory entities.

18.9 Certificates of Confidentiality

An application for human participant approval has been submitted to the UMMS IRB, the applicant organization, and the WHREC, for the Aurum Tembisa CRC and Isango Lethemba TB research Unit.

18.10 Critical Event Reporting

Critical events (CE) include the following classes:

- Unanticipated Problems
- Serious or Continuing Noncompliance
- Suspension or Termination of EC/IRB Approval
- Suspected Research Misconduct

The METHOD trial will report AEs to WHREC and SAHPRA as specified in SOP-IEC-005v6, Adverse Events Processing (Appendix E) and in the SAHPRA document Reporting Adverse Drug Reactions in South Africa version 1_2 (Appendix F), respectively. The following documents will be provided:

- Protocol related documents (e.g., protocol number, ICFs)
- Copies of applicable source documents, CRFs, or other materials that provide details about the incident
- Any other available information that will help determine if a critical event has occurred

Unanticipated Problems

- Unanticipated Problems (UPs) are defined by having these three characteristics:
- The adverse event is unexpected

- A reasonable possibility that the adverse event is related to participation in the study
- The adverse event suggests that the research places participants or others at a greater risk of harm than was previously known or recognized

The PI/IoR or designee will notify WHREC and SAHPRA when a UP involving risk to participants or others has occurred, in accordance with policies of the participating institutions. In addition, all SUSARs will be reported to the DAIDS MO within 5 days of being reported to the site. The PI or designee will determine if immediate suspension of some (e.g., suspend enrollment for new participants) or all research activities is warranted while the UP is being evaluated. The PI or designee will determine if immediate suspension of some (e.g., suspend enrollment for new participants) or all research activities is warranted while the UP is being evaluated. The PI/IoR or designee will follow the actions directed by WHREC, SAHPRA, and/or other relevant entity. Timely updates will be provided to the appropriate WHREC and SAHPRA staff members on all follow-up reports and communications. A follow-up or final report will be submitted within 15 calendar days that he/she becomes aware of the event and continue to provide updates until the critical event issue is resolved.

The EC/IRBs of all participating institutions will review reports of UPs involving risks to participants or others. They will approve corrective actions per institutional policy and ensure that these actions are implemented to protect the rights and welfare of all participants and remediate the situation.

The awardee institution (UMMS) will ensure the NIAID Clinical Terms of Award and Terms of the FWA for the Protection of Human Subjects are met. The institution will ensure there is prompt reporting of the UP to WHREC, SAHPRA

, the EC/IRBs of all participating institutions, the DAIDS PO and MO, and applicable regulatory entities, including OHRP and FDA, when appropriate. The awardee institution will assure that appropriate actions have been taken by all subawardees (Aurum, SlgN UCT, and Port Elizabeth) and that required reports have been sent to applicable regulatory entities.

Serious or Continuing Noncompliance

When serious or continuing noncompliance has occurred, the PI/IoR or designee will provide an initial report to WHREC and SAHPRA as soon as possible, but no later than 3 reporting days after he/she becomes aware of the noncompliance. He/she will determine if immediate suspension of some (e.g., suspend enrollment for new participants) or all research activities is warranted while the noncompliance is being evaluated. The PI/IoR or designee will follow the actions directed by WHREC, SAHPRA or other relevant entity. Timely updates will be provided to the DAIDS PO and MO, or designee on all follow-up reports and communications. The PI/IoR or designee will submit a follow-up or final report within 15 calendar days of becoming aware of the noncompliance and continue to provide updates until the critical issue is resolved. The report(s) will include all the information related to the noncompliance that is available at the time, including corrective actions.

The WHREC and/or SAHPRA will determine when serious or continuing noncompliance has occurred or review noncompliance reports from other entities. The WHREC and/or SAHPRA will approve corrective actions per institutional policy and ensure that these actions are implemented to protect the rights and welfare of all participants and remediate the situation.

The awardee institution (UMMS) will ensure the NIAID Clinical Terms of Award and Terms of the FWA for the Protection of Human Subjects are met. The awardee institution will ensure there is prompt reporting of the serious or continuing noncompliance to their EC/IRB and appropriate institutional officials, the DAIDS PO and MO, and applicable regulatory entities, including OHRP and FDA, when appropriate. The awardee institution will assure that appropriate actions have been taken by all subawardees and that required reports have been sent to applicable regulatory

entities. The subawardee institutions will ensure: that the terms of the FWA for the Protection of Human Subjects are met; that there is prompt reporting of the serious or continuing noncompliance to the local EC/IRB and other institutional officials per its institutional policy; and that the awardee institution is informed of the serious or continuing noncompliance and actions taken.

Suspension or Termination of EC/IRB Approval

The EC/IRB will promptly notify the investigator and institutional officials in writing of any suspension or termination of its approval, including the reason(s) for this action. The EC/IRB will further approve corrective actions to ensure that the rights and welfare of all participants are protected and to remediate the situation.

The awardee institution (UMMS) will ensure the NIAID Clinical Terms of Award and Terms of the FWA for the Protection of Human Subjects is met. The institution will ensure there is prompt reporting of the suspension or termination of EC/IRB approval to appropriate institutional officials, the DAIDS PO and MO, OHRP, and FDA (when applicable). The awardee institution will further assure that appropriate actions have been taken by all subawardees and that required reports have been sent to applicable regulatory entities, including OHRP and FDA, when applicable. The subawardee institutions will ensure the Terms of the Federalwide Assurance (FWA) for the Protection of Human Subjects are met. The subawardee institutions will ensure: that there is prompt reporting of the suspension or termination of EC/IRB approval to the local EC/IRB and other institutional officials per its institutional policy; and that the awardee institution is informed of the suspension or termination of IRB/EC approval and actions taken.

The PI/IoR or designee will notify DAIDS as soon as possible, but no later than 3 reporting days after becoming aware of the suspension or termination of EC/IRB approval, through the DAIDS Protocol Registration System (DPRS) if the EC/IRB suspends or terminates part or all of the research activities for the study. The PI/IoR will: notify the DAIDS PO or COR directly if the study is not registered through the DPRS; report the EC/IRB actions to the relevant protocol team and network leadership; forward a copy of all correspondence from the EC/IRB pertaining to the suspension or termination, including corrective actions; follow the action(s) as directed by the IRB/EC, DAIDS, or other relevant entity; and provide timely updates to DAIDS on all follow-up reports and communications. Research will not be resumed unless and until the PI/IoR receives written communication from the EC/IRB that it has reapproved the study, and concurrence from DAIDS.

Suspected Research Misconduct

The awardee institution (UMMS) will investigate whether an allegation of fabrication, falsification, or plagiarism is research misconduct. The institution will coordinate the handling of research misconduct allegations originating at the subawardee's site. Research misconduct records will be retained in accordance with Federal guidelines and regulations.

The awardee institution will retain research misconduct records, meeting the requirements of DAIDS Policy on Storage and Retention of Clinical Research Records per Federal guidelines and regulations (PHS 42 CFR 93.223 & 93.224).

Site staff, including site personnel and the PI/IoR or designee will follow the institution's policy, procedure, and process for reporting allegations of research misconduct, including protecting the confidentiality of the person(s) making an allegation of research misconduct, the person(s) against whom the allegation of research misconduct is directed, and the study participants. Site personnel will report allegations of research misconduct to the PI and/or designee within 24 hours of

becoming aware of the incident. If site personnel cannot notify the PI/IoR or designee for fear of retribution or another reason, the site personnel will notify an appropriate person within their institution and DAIDS. The PI/IoR or designee will notify the institutional Research Integrity Officer, the EC/IRB, and the appropriate DAIDS staff member of all applicable, known facts about the allegation. The PI/IoR or designee will review the available information and assess whether the allegation of research misconduct represents an additional class of Critical Event and will determine if immediate suspension of some (e.g., suspend enrollment for new participants) or all research activities is warranted while the allegation is being evaluated.

The institution's Research Integrity Officer and/or designee will follow NIH and institutional policies when assessing the allegation to determine whether the allegation:

- Meets the definition of research misconduct
- Involves PHS23 supported research, research training, or research records
- Is sufficiently credible and specific so that potential evidence of research misconduct may be identified

The institution's Research Integrity Officer will ensure that the EC/IRB has been notified of the research misconduct allegation and any decision to open an investigation. It is the responsibility of the PI/IoR to inform DAIDS of the institution's interim and final actions in response to the investigation of research misconduct allegations.

Critical Event Reporting

For unanticipated problems involving risks to participants or others, serious or continuing noncompliance, or suspension or termination of EC/IRB approval, a report submitted to DAIDS will include the following:

- Name of the institution conducting the research
- Title of the research project in which the problem, noncompliance or suspension/termination occurred
- Name of the PI and IoR on the protocol
- Research project number assigned by the ECIRB and the number of NIAID/DAIDS award
- A detailed description of the critical event
- A description of the plans and/or actions taken by the institution to address the critical event

18.11 Communicable Disease Reporting

Participants in the METHOD trial will be recruited from public health clinics and those enrolled will have documented active TB disease and HIV infection. This information will be known to the local public health authorities.

18.12 Incidental Findings

There is potential for incidental findings (IF) concerning individual participants METHOD trial since the planned clinical evaluations, including physical examinations, clinical laboratory tests, chest X-rays and pulmonary function tests far exceed the standard of usual care for TB/HIV patients in the local community. If such findings are determined by the Aurum Tembisa CRC and Isango Lethemba TB Research Unit, medical staff, per the ICF, participants have the option to choose to be informed about these findings or not, which may possibly warrant additional investigation or treatment. Those participants who opt-in will be notified without delay and, with permission from the participant, the CRC staff will ensure referral to the appropriate community provider (e.g., public health clinic) as indicated by the nature of the incidental finding.

Research laboratory evaluations are purely investigational in nature with no basis in evidence or regulatory approval to suggest any change in clinical management. The only use of participant DNA samples would be for epigenetic studies and pharmacogenomics analysis of a restricted set of genes associated with metformin disposition. None of these targets are implicated in genetic diseases and data on polymorphisms in these particular genes is not recognized as contributing to medical management.

18.13 New Findings

New findings that, in the assessment of the Principal Investigator/Investigator of Record (PI/IoR), might impact the willingness of an enrolled participant to continue in the METHOD trial will be conveyed in writing to the participant. Such new findings will also be discussed in person with the affected participant. If, in the judgement of the PI/IoR, the new finding does pose increased potential risk to the participant, then the study drug will be discontinued. If, in the judgement of the PI/IoR, the new finding does not pose an increased potential risk, the study drug may be continued. In either case, the participant will be informed of the option to withdraw from the study. The PI/IoR will notify the EC/IRB of any new findings that may impact the willingness of individual to enroll in the study or enrolled individuals to continue in the study. The EC/IRB will determine whether revisions in the ICF(s) are required, and whether participants already enrolled in the study will be required to re-consent using a revised, approved ICF(s).

18.14 Study Discontinuation

The study may be discontinued at any time by the EC/IRB, SAHPRA, NIAID, or other government entities as part of their duties to ensure that research participants are protected. Termination could occur after the development of unacceptable toxicities or as a recommended outcome of a Data Safety Monitoring Board (DSMB) review.

18.15 Post-Trial Access

Post-trial access is not applicable for the omics control group. For the intervention group, metformin is approved worldwide for the treatment of type 2 DM. It is commonly used off-label for other indications including pre-diabetes, polycystic ovary syndrome and HIV-associated lipodystrophy and metabolic syndrome. While there is no limitation for post-trial access to this drug, results from the METHOD trial will not provide a sufficient basis in evidence to justify its use as HDT for TB. It is anticipated that positive results of the METHOD trial would provide a rational basis and motivation to conduct a Phase 3 clinical trial to confirm its efficacy for adjunctive treatment of TB.

18.16 Ancillary Benefits

Participants will receive treatment for TB and HIV through the local public health clinic system during the period of their involvement in the METHOD trial. Medical staff at the Aurum Tembisa CRC and Isango Lethemba TB Research Unit will work with these and other local authorities to provide ancillary care, if needed, for study participants.

18.17 Community Advisory Group and Other Stakeholders

The Aurum Tembisa CRC and Isango Lethemba TB Research Unit operates with a Community Engagement Unit, study coordinators, social workers, field workers/counsellors and 4 interns. This unit promotes social mobilization (door-to-door campaigns, community outreach events including HIV commemorative events) and education (onsite training and community briefings). A 20-member Community Advisory Group (CAG) was established in July 2011, for the Aurum Tembisa CRC. The CAG includes people living with HIV/AIDS, ex-TB patients, HAST coordinators and Clinic Heads, among others. The remit of the CAG is to:

- Enhance transparency and bridge the gap between Aurum and the community
- Represent and advocate for the needs and interest of the community
- Ensure that the community has a voice in decision-making that affects their health
- Advise on the best ways to implement health programs and conducting clinical trials

The CAG will be involved in the planning and implementation of the METHOD trial to ensure that the community is educated as to the study aims, plans, risks and benefits.

19 ADMINISTRATIVE PROCEDURES

Administrative procedures include EC/IRB submission and approvals, protocol registration, regulatory oversight, protocol modifications and implementation, and registration in ClinicalTrials.gov.

19.1 Protocol Registration

The Aurum Tembisa CRC and Isango Lethemba TB Research Unit, are network study sites. The Protocol Chair (Prof. Robert Wallis) will ensure that the site adheres to the Protocol Registration Policy. The compliance of the site with NIAID (DAIDS) policies, EC/IRB requirements, regulatory documents, and U.S federal regulations for investigational research with human participants are tracked through the protocol registration system. The system also confirms that informed consent forms comply with US regulations and guidelines.

The DAIDS Learning Portal includes training on the DAIDS Protocol Registration (PR) Policy and Manual, on the web-based system (DPRS) and is used to submit PR documents. The METHOD trial staff responsible for preparing and submitting PR documents and those involved with reviewing or tracking PR documents will attend the training.

Protocol Registration Training

Staff will attend the training in order to use the DPRS web-based system and to comply with the PR policy. Aurum regulatory will submit PR material through the DPRS. Prior to implementing the protocol and enrolling participants, the sites will receive approval to conduct the study from the EC/IRBs and other regulatory entities, complete the PR process with the DAIDS Protocol Registration Office (DAIDS PRO), and receive a registration notification from the DAIDS PRO indicating successful completion of the initial PR process. If notification of disapproval is received, corrective materials will be resubmitted to the DAIDS PRO. The sites will begin enrollment once it is notified by the DAIDS Program Officer or Contracting Officer.

Protocol Registration Submission and Modifications

After receiving the final approval of the protocol and informed consent documents from the EC/IRBs and other regulatory entities, the IoR and other MPIs will ensure that Aurum regulatory submits all the required protocol registration documents to the DAIDS PRO and all protocol registration requirements are met, as specified in the Protocol Registration manual. Requirements for the protocol registration will be decided during the review of the protocol at the time of the DAIDS SRC assessment or regulatory review. All final decisions regarding modifications of the PR process made by the DAIDS PRT and DAIDS staff will be documented and be shared with the DAIDS staff and the Protocol Team/Protocol PI/Grant PI/Contract PI.

Aurum regulatory will submit the DAIDS-approved amended protocol and ICFs to the UMMS IRB in the US within 45 calendar days and non-US EC/IRBs within 75 days. They will also submit the noted materials to SAHPRA. If the timeline cannot be met, the justification will be provided with the registration packet submission to the DAIDS PRO and a copy kept in the regulatory file. Once approved, the amended protocol and ICFs will be implemented immediately. A final registration notification from the DAIDS PRO is not required prior to implementing an amendment.

Aurum regulatory will submit the required EC/IRB and SAHPRA approved amendment registration documents to the DAIDS PRO within 14 calendar days after receipt of all final written documentation of EC/IRB and SAHPRA approval for the amendment. The documentation will include the date that the amended protocol and ICFs were submitted to the EC/IRBs and

SAHPRA. The Aurum Tembisa CRC and Isango Lethemba TB Research Unit will document the date that they received EC/IRB/SAHPRA approvals in the site's regulatory files. The DAIDS PRO will notify (registration notification) regulatory team that it has successfully completed the amendment protocol registration process.

Aurum regulatory will complete the protocol registration process, approved by the DAIDS SRC. Upon receiving EC/IRB/SAHPRA approvals, the Aurum regulatory will submit all required protocol registration documents to the DAIDS PRO through the DAIDS PR System. The Aurum Tembisa CRC and Isango Lethemba TB Research Unit will place a copy of all final registration notifications from the DAIDS PRO in the site's regulatory files.

Once DAIDS approves a letter of amendment (LoA), the Aurum regulatory will submit the LoA and other required materials within 45 calendar days to the UMMS IRB and 75 calendar days to the non US EC/IRBs from the date the LoA was approved by DAIDS. If the Aurum regulatory team is unable to submit the LoA within the designated timeline to the UMMS IRB or the non US EC/IRBs, the Aurum regulatory team will provide a justification with the packet submission to the DAIDS PRO and file a copy in the regulatory file.

Once the IRB approval is received, the LoA and revised ICF(s) will be implemented immediately. Aurum regulatory will submit the required EC/IRB and other applicable regulatory entities approved amendment registration documents to the DAIDS PRO within 14 calendar days after receipt of all final written documentation of IRB/EC/approving entity approval for the amendment. The Aurum Tembisa CRC and Isango Lethemba TB Research Unit will document the date of EC/IRB approval in the site's regulatory files. DAIDS PRO will notify Aurum regulatory indicating successful completion of the LoA registration process.

Continuing Review/Annual Review

The Aurum regulatory team will submit the required EC/IRB approved Continuing/Annual Review documentation within 14 calendar days after receipt of final written documentation from the EC/IRB. The date of approval will be documented in the site's regulatory files. Once submitted to the DAIDS PRO, the Aurum regulatory team will receive confirmation of submission and review.

Change of IoR

The Investigator of Record (IoR) is listed on item 1 on form 1572 or the DAIDS IoR form. If there is a change in the IoR, the Aurum Tembisa CRC and Isango Lethemba TB Research Unit will submit the required documentation to the DAIDS PRO to officially change the IoR for a protocol. The documentation will be done within 30 calendar days from the time of notification that the current IoR will no longer serve as the IoR for the study. The change of IoR is not official until the Aurum Tembisa CRC and/or Isango Lethemba TB Research Unit receives the Approval notification for the change of IoR from the DAIDS PRO.

Deregistration

The Aurum regulatory team will submit a request for deregistration to the DAIDS PRO once the study is completed. Once it has been deregistered, the Aurum Tembisa CRC will receive a Deregistration Notification from the DAIDS PRO. The Aurum Tembisa CRC and Isango Lethemba TB Research Unit are not considered deregistered from a study until this notification is received. Upon completion of the deregistration process for a protocol, the Aurum Tembisa CRC and Isango Lethemba TB Research Unit will no longer receive safety information from DAIDs related to the protocol.

19.2 Regulatory Oversight

Regulatory oversight for the METHOD trial rests with DAIDS, including the decision on whether and IND will be submitted. Within South Africa, SAHPRA is responsible for oversight of compliance with national regulations.

The Aurum regulatory team will complete the protocol registration process for all clinical research sponsored by DAIDS that is reviewed by DAIDS Scientific Review Committees, namely the Prevention Sciences Review Committee (PSRC) and the Clinical Sciences Review Committee (CSRC) and if it is determined protocol Registration is required. Upon receiving final EC/IRB and other applicable approving entity approvals, the Aurum regulatory team will submit all required registration documents to the DAIDS PRO via the DAIDS DPRS

19.3 Study Implementation

The Study will be implemented according to the clinical protocol and the Standard Operating Procedures SOPs. Procedures and operations will be revised as necessary to comply with the requirements of DAIDS, the EC/IRB, and SAHPRA.

The METHOD trial will use existing SOPs with project-specific revisions whenever possible. Prior to the initiation of this trial, the following SOPS are required:

- SOP Development and Version Control
- Personnel Qualifications
- Personnel Training and Certification Documentation
- Site Quality Management Plan (QMP)
- Essential Documents
- Source Documents
- Reporting Adverse Events
- Reporting Expedited Adverse Events (EAES) or SAEs to DAIDS
- Confidential HIV Counselling and Testing Procedure
- Unblinding for Safety
- Laboratory SOPs
 - Biohazard Safety and Containment and Occupational Safety
 - Laboratory Data Management and Storage
 - Lab Quality Management Plan
 - Specimen Acquisition, Processing, Tracking and Storage
 - Lost, Broken, and Leaking Samples
 - Receipt and Processing all Samples
 - Specimen Transport
 - Shipping Specimens Locally
 - Shipping Specimens Internationally
- Computer Room, Hardware, and Data Security (including system user account maintenance)
- Data Acquisition, Entry, and Processing
- Data Queries and Data Entry Correction
- Data Collection Training
- Data Quality Management
- Data Storage
- Pharmacy Operations SOPS

-
- Protocol Specific Pharmacy SOPS
 - Specific Requirements for a Particular Agent
 - Regimens and Administration
 - Protocol Specific Prescriptions
 - Monitoring SOPS
- Review and Follow-up of Monitoring Report Findings

19.4 ClinicalTrials.gov

This protocol is subject to the Food and Drug Administration Amendments Act of 2007 (FDAAA) and will be registered in ClinicalTrials.gov.

20 PUBLICATION POLICY

Following completion of the study, the investigators plan to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This trial will be registered in ClinicalTrials.gov before patient enrollment is initiated. It is anticipated that the results of this study will be submitted for publication in a peer-reviewed scientific journal. Authorship will be extended to the following individuals: study investigators, site PIs, and other individuals having major contribution to the study design, implementation, data analysis, and preparation of the written manuscript.

Prior to submission for presentation or publication, any materials derived wholly or in part from this study must be submitted to the study PIs, site PI, and all co-authors for review.

21 REFERENCES

1. World Health Organization. Global Tuberculosis Report 2015. 2015. Geneva, WHO Press.
2. Johnson, J.L., D.J. Hadad, R. Dietze, E.L. Maciel, B. Sewali, P. Gitta, A. Okwera, R.D. Mugerwa, M.R. Alcaneses, M.I. Quelapio, T.E. Tupasi, L. Horter, S.M. Debanne, K.D. Eisenach, and W.H. Boom. 2009. Shortening treatment in adults with noncavitary tuberculosis and 2-month culture conversion. *Am. J. Respir. Crit Care Med.* 180:558-563.
3. Jindani, A., T. S. Harrison, A. J. Nunn, P. P. Phillips, G. J. Churchyard, S. Charalambous, M. Hatherill, H. Geldenhuys, H. M. McIlleron, S. P. Zvada, S. Mungofa, N. A. Shah, S. Zizhou, L. Magweta, J. Shepherd, S. Nyirenda, J. H. van Dijk, H. E. Clouting, D. Coleman, A. L. Bateson, T. D. McHugh, P. D. Butcher, and D. A. Mitchison. 2014. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. *N. Engl. J. Med.* 371:1599-1608.
4. Gillespie, S.H., A.M. Crook, T.D. McHugh, C.M. Mendel, S.K. Meredith, S.R. Murray, F. Pappas, P.P. Phillips, and A.J. Nunn. 2014. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. *N. Engl. J. Med.* 371:1577-1587.
5. Merle, C.S., K. Fielding, O.B. Sow, M. Gninafon, M.B. Lo, T. Mthiyane, J. Odhiambo, E. Amukoye, B. Bah, F. Kassa, A. N'Diaye, R. Rustomjee, B.C. de Jong, J. Horton, C. Perronne, C. Sismanidis, O. Lapujade, P.L. Olliaro, and C. Lienhardt. 2014. A four-month gatifloxacin-containing regimen for treating tuberculosis. *N. Engl. J. Med.* 371:1588-1598.
6. Pasipanodya, J.G., S.J. McNabb, P. Hilsenrath, S. Bae, K. Lykens, E. Vecino, G. Munguia, T. L. Miller, G. Drewyer, and S.E. Weis. 2010. Pulmonary impairment after tuberculosis and its contribution to TB burden. *BMC Public Health* 10:259.
7. Willcox, P.A. and A.D. Ferguson. 1989. Chronic obstructive airways disease following treated pulmonary tuberculosis. *Respir. Med* 83:195-198.
8. Hoger, S., K. Lykens, S.F. Beavers, D. Katz, and T. L. Miller. 2014. Longevity loss among cured tuberculosis patients and the potential value of prevention. *Int. J. Tuberc. Lung Dis.* 18:1347-1352.
9. Shuldiner, J., A. Leventhal, D. Chemtob, and Z. Mor. 2016. Mortality after anti-tuberculosis treatment completion: results of long-term follow-up. *Int. J. Tuberc. Lung Dis.* 20:43-48.
10. Abdool Karim, S.S., G.J. Churchyard, Q.A. Karim, and S.D. Lawn. 2009. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. *Lancet* 374:921-933.
11. Ehrlich, R.I., N. White, R. Norman, R. Laubscher, K. Steyn, C. Lombard, and D. Bradshaw. 2004. Predictors of chronic bronchitis in South African adults. *Int. J. Tuberc. Lung Dis.* 8:369-376.
12. Wallis, R.S. and R. Hafner. 2015. Advancing host-directed therapy for tuberculosis. *Nat. Rev. Immunol.* 15:255-263.
13. Singhal, A., L. Jie, P. Kumar, G.S. Hong, M.K. Leow, B. Paleja, L. Tsenova, N. Kurepina, J. Chen, F. Zolezzi, B. Kreiswirth, M. Poidinger, C. Chee, G. Kaplan, Y.T. Wang, and G. De Libero. 2014. Metformin as adjunct antituberculosis therapy. *Sci. Transl. Med.* 6:263ra159.
14. Dutta, M.K., M.L. Pinn, P.C. Karakousis. 2017. Metformin adjunctive therapy does not improve the sterilizing activity of the first-line antitubercular regimen in mice. *Antimicrob. Agents Chemother.* 61:e00652-17.
15. Lachmandas, E., C. Eckold, J. Bohme, V.A.C.M. Koeken, M.B. Marzuki, R.J.W. Arts, J. Chen, K.W.W. Teng, J. Ratter, J. Ratter, E.J. Smolders, C. Van den Heuvel, R. Stienstra, H.M. Dockrell, E. Newell, M.G. Netea, A. Singhal, J.M. Cliff, R. Van Crevel. 2019. Metformin alters human host responses to *Mycobacterium tuberculosis* in healthy subjects. *J. Infect. Dis.* 220:139-150.
16. Leow, M.K.S., R. Dalan, C.B.E. Chee, A. Earnest, D.E.K. Chew, A.W.K. Tran, W.Y.C. Kon, M. Jong, T. Barkham, and Y.T. Wang. 2014. Latent tuberculosis in patients with diabetes

- mellitus: Prevalence, progression and public health implications. *Clin. Endocrinol. Diabetes*. 122:528-532.
17. Maruparu, S., P. Senapati, S. Pathadka, S.S. Miraj, M.K. Unnikrishnan, and M.K. Manu. 2017. Protective effect of metformin against tuberculosis infections in diabetic patients: an observational risk study of south Indian tertiary healthcare facility. *Braz. J. Infect. Dis*. 21:312-316.
18. Degner, N.R., J.-Y. Wang, J.E. Golub, and P.C. Karakousis. 2017. Metformin use reverses the increased mortality associated with diabetes mellitus during tuberculosis treatment. *Clin. Infect. Dis*. 66:198-205.
19. Pan, S.-W., Yen, Y.-F., Kou, Y.R., Chuang, P.-H. Su, V.Y.-F., Feng, J.-Y., Chan, Y.-J., and Su, Y.-J. 2018. The risk of tuberculosis disease in type 2 diabetes patients initiating metformin versus sulfonylureas. *Chest*. In press.
20. Santos, A., D. Lavagnoli, G. Amorim, J. Oliveira, S. Riva, and F. Mello. 2017. The effect of metformin on smear and culture conversion of diabetic patients with tuberculosis. *Am. J. Respir. Crit. Care. Med*. 195:A2110.
21. Lee, Y.J., S.K. Han, J.H. Park, J.K. Lee, D.K. Kim, H.S. Chung, and E.Y. Heo. 2018. The effect of metformin on culture conversion in tuberculosis patients with diabetes mellitus. *Korean J. Intern. Med*. doi: 10.3904/kjim.2017.249. [Epub ahead of print].
22. Ma, Y., Y. Pang, W. Shu, Y.H. Liu, Q.P. Ge, J. Du, L. Li, W.W. Gao. 2018. Metformin reduces the relapse rate of tuberculosis patients with diabetes mellitus: experiences from 3-year follow-up. *Eur. J. Clin. Microbiol. Infect. Dis*. doi: 10.1007/s10096-018-3242-6. [Epub ahead of print].
23. Lin, S.Y., H.P. Tu, P.L. Lu, T.C. Chen, W.H. Wang, I.W. Chong, Y.H. Chen. 2018. Metformin is associated with a lower risk of active tuberculosis in patients with type 2 diabetes. *Respirology* 23:1063-1073.
24. Tseng, C.H. 2018. Metformin decreases the risk of tuberculosis in type 2 diabetes patients. *J. Clin. Med*. 7:264.
25. Magee, M.J., A.D. Salindri, H. Kornfeld, A. Singhal. 2019. Reduced prevalence of latent tuberculosis infection in diabetes patients using metformin and statins. *Eur. Respir. J*. 53: 1801695.
26. Elkington, P.T., C.A. Ugarte-Gil, J.S. Friedland. 2011. Matrix metalloproteinases in tuberculosis. *Eur. Respir. J*. 38:456-64.
27. Kumar, N.P., K. Moideen, V. Viswanathan, B.S. Shruthi, S. Sivakumar, P.A. Menon, H. Kornfeld, S. Babu. 2018. *BMC Infect. Dis*. 18:345.
28. Mave, V., S. Gaikwad, M. Barthwal, R. Lokhande, D. Kadam, S. Dharmshale, R. Bharadwaj, A. Kagal, N. Pradhan, S. Deshmukh, S. Atre, T. Sahasrabudhe, S. Meshram, A. Kakrani, V. Kulkarni, S. Raskar, N. Suryavanshi, H. Kornfeld, K.E. Dooley, S. Chon, A. Gupte, A. Gupta, N. Gupte, J.E. Golub. Mortality during tuberculosis treatment increases substantially among tuberculosis patients with diabetes mellitus, and particularly among patients not receiving metformin. (Submitted for publication).
29. Doyle, M.-A., J. Singer, T. Lee, M. Muir, C. Cooper. 2016. Improving treatment and liver fibrosis outcomes with metformin in HCV-HIV coinfectd and HCV mono-infected patients with insulin resistance: study protocol for a randomized controlled trial. *Trials* 17:331.
30. Shih, C.J., Y.L. Wu, P.W. Chao, S.C. Kuo, C.Y. Yang, S.Y. Li, S.M. Ou, Y.t. Chen. 2015. Association between use of oral anti-diabetic drugs and the risk of sepsis: a nested case-control study. *Sci. Rep*. 5:15260.
31. Eliakim-Raz, N, G. Fishman, D. Yahav, E. Goldberg, G.Y. Stein, H.B. Zvi, A. Barsheshet, J. Bishara. 2015. Predicting clostridium difficile infection in diabetic patients and the effect of metformin therapy: a retrospective, case-control study. *Eur. J. Clin. Microbiol. Infect. Dis*. 15:1201-1205.
32. Cameron, A.R., V.L. Morrison, D. Levin, M. Mohan, C. Forteath, C. Beall, A.D. McNeilly, D.J. Balfour, T. Savinko, A.K. Wong, B. Viollet, K. Sakamoto, S.C. Fagerholm, M. Foretz,

- C.C. Lang, G. Rena. 2016. Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ. Res.* 119:652-665.
33. Prada-Medina, C.A., K.F. Fukutani, N. Pavan Kumar, L. Gil-Santana, S. Babu, F. Lichtenstein, K. West, S. Sivakumar, P.A. Menon, V. Viswanathan, B.B. Andrade, H.I. Nakaya, H. Kornfeld. 2017. Systems immunology of diabetes-tuberculosis comorbidity reveals signatures of disease complications. *Sci. Rep.* 7:1999.
34. Brima, W., D.J. Eden, S.F. Mehdi, M. Bravo, M.M. Wiese, J. Stein, V. Almonte, D. Zhao, I.I. Kurland, J.E. Pessin, T. Zima, H.B. Tanowitz, L.M. Weiss, J. Roth, F. Nagajyothi. The brighter (and evolutionarily older) face of the metabolic syndrome: evidence from *Trypanosoma cruzi* infection in CD-1 mice. 2015. *Diabetes Metab. Res. Rev.* 31:346-359.
35. Othman, A.A., D.M. Rayia, D.S. Ashour, E.M. Saied, D.H. Zineldeen, A.A. El-Ebiary. 2016. Atorvastatin and metformin administration modulates experimental *Trichinella spiralis* infection. *Parasitol. Int.* 65:105-112.
36. Shirazi, F., D. Farmakiotis, Y. Yan, N. Albert, K.A. Do, D.P. Kontoyiannis. 2014. Diet modification and metformin have a beneficial effect in a fly model of obesity and mucormycosis. *PLoS One* 9: e108635.
37. Tzanavar, I.T., A. Varela, S. Theocharis, E. Ninou, A. Kapelouzou, D.V. Cokkinos, M.I. Kontaridis, K.P. Karalis. 2016. Metformin protects against infection-induced myocardial dysfunction. *Metabolism* 65:1447-1458.
38. Xun, Y.H., Y.J. Zhang, Q.C. Pan, R.C. Mao, Y.L. Qin, H.Y. Liu, Y.M. Zhang, Y.S. Yu, Z.H. Tang, M.J. Lu, G.Q. Zang, J.M. Zhang. 2014. Metformin inhibits hepatitis B virus protein production and replication in human hepatoma cells. *J. Viral Hepat.* 21:597-603.
39. Patkee, W.R., G. Carr, E.H. Baker, D.L. Baines, J.P. Garnett. 2018. Metformin prevents the effects of *Pseudomonas aeruginosa* on airway epithelial tight junctions and restricts hyperglycaemia-induced bacterial growth. *J. Cell Mol. Med.* 20:758-764.
40. Kang, W., T. Wang, Z. Hu, F. Liu, Y. Sun, S. Ge. 2017. Metformin inhibits *Porphyromonas gingivalis* lipopolysaccharide-influenced inflammatory response in human gingival fibroblasts via regulating activating transcription factor-3 expression. *J. Periodontol.* 88:e169-e178.
41. Tornio, A., M. Niemi, P. J. Neuvonen, and J.T. Backman. 2012. Drug interactions with oral antidiabetic agents: pharmacokinetic mechanisms and clinical implications. *Trends Pharmacol. Sci.* 33:312-322.
42. Padmapriyadarsini, C., P.K. Bhavani, M. Natrajan, C. Ponnuraja, H. Kumar, S.N. Gomathy, R. Guleria, S.M. Jawahar, M. Singh, T. Balganes, S. Swaminathan. 2019. Evaluation of metformin in combination with rifampicin containing antituberculosis therapy in patients with new, smear-positive pulmonary tuberculosis (METRIF): study protocol for a randomised clinical trial. *BMJ Open* 9:e024363.
43. Pryor, R. and F. Cabreiro. 2015. Repurposing metformin: an old drug with new tricks in its binding pockets. *Biochem. J.* 471:307-322.
44. Hadigan, C., C. Corcoran, N. Basgoz, B. Davis, P. Sax, and S. Grinspoon. 2000. Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. *J. Am. Med. Assoc.* 284:472-477.
45. Fitch, K., S. Abbara, H. Lee, E. Stavrou, R. Sacks, T. Michel, L. Hemphill, M. Torriani, and S. Grinspoon. 2012. Effects of lifestyle modification and metformin on atherosclerotic indices among HIV-infected patients with the metabolic syndrome. *AIDS* 26:587-597.
46. Srinivasa, S., K. Wong, K. V. Fitch, J. Wei, E. Petrow, A. M. Cypess, M. Torriani, and S. K. Grinspoon. 2015. Effects of lifestyle modification and metformin on irisin and FGF21 among HIV-infected participants with the metabolic syndrome. *Clin. Endocrinol. (Oxf)* 82:678-685.
47. van Wijk, J.P., A.I. Hoepelman, E.J. de Koning, G. Dallinga-Thie, T.J. Rabelink, and M.C. Cabezas. 2011. Differential effects of rosiglitazone and metformin on postprandial lipemia in patients with HIV-lipodystrophy. *Arterioscler. Thromb. Vasc. Biol.* 31:228-233.

48. Diehl, L.A., B.A. Fabris, D.S. Barbosa, E.C. De Faria, S.L. Wiechmann, and A.J. Carrilho. 2008. Metformin increases HDL3-cholesterol and decreases subcutaneous truncal fat in nondiabetic patients with HIV-associated lipodystrophy. *AIDS Patient Care STDS*. 22:779-786.
49. Kohli, R., A. Shevitz, S. Gorbach, and C. Wanke. 2007. A randomized placebo-controlled trial of metformin for the treatment of HIV lipodystrophy. *HIV. Med.* 8:420-426.
50. Mulligan, K., Y. Yang, D.A. Wininger, S.L. Koletar, R.A. Parker, B.L. Alston-Smith, J.T. Schouten, R.A. Fielding, M.T. Basar, and S. Grinspoon. 2007. Effects of metformin and rosiglitazone in HIV-infected patients with hyperinsulinemia and elevated waist/hip ratio. *AIDS* 21:47-57.
51. Coll, B., J.P. van Wijk, S. Parra, C.M. Castro, I.M. Hoepelman, C. Alonso-Villaverde, E.J. de Koning, J. Camps, N. Ferre, T. J. Rabelink, M. Tous, and J. Joven. 2006. Effects of rosiglitazone and metformin on postprandial paraoxonase-1 and monocyte chemoattractant protein-1 in human immunodeficiency virus-infected patients with lipodystrophy. *Eur. J. Pharmacol.* 544:104-110.
52. Thierry Saint-Marc, T., Touraine, J.L. 1999. Effects of metformin on insulin resistance and central adiposity in patients receiving effective protease inhibitor therapy. *AIDS* 13:1000-1002.
53. Tomazic, J., Karner, P., Vidmar, L., Maticic, M., Sharma, P.M., Janez, A. 2005. Effect of metformin and rosiglitazone on lipid metabolism in HIV infected patients receiving protease inhibitor containing HAART. *Acta Dermatoven. APA.* 14:99-105.
54. Sheth, S.H. and R.J. Larson. 2010. The efficacy and safety of insulin-sensitizing drugs in HIV-associated lipodystrophy syndrome: a meta-analysis of randomized trials. *BMC Infect. Dis.* 10:183.
55. Kornfeld, H., K. West, K. Kane, S. Kumpatla, R. R. Zacharias, C. Martinez-Balzano, W. Li, and V. Viswanathan. 2016. High prevalence and heterogeneity of diabetes in patients with TB in South India: A report from the effects of diabetes on tuberculosis severity (EDOTS) Study. *Chest* 149:1501-1508.
56. Schwartz, S., V. Fonseca, B. Berner, M. Cramer, Y.K. Chiang, and A. Lewin. 2006. Efficacy, tolerability, and safety of a novel once-daily extended-release metformin in patients with type 2 diabetes. *Diabetes Care.* 29: 759-764.
57. Meintjes, G., M.A. Moorhouse, S. Carmona S, N. Davies, S. Dlamini, C. van Vuuren, T. Manzini, M. Mathe, Y. Moosa, J. Nash, J. Nel, Y. Pakade, J. Woods, G. Van Zyl, F. Conradie, F. Venter. 2017. Adult antiretroviral therapy guidelines 2017. *S. Afr. J. HIV Med.* 18:776.
58. Boeree, M.J., N. Heinrich, R. Aarnoutse, A.H. Diacon, R. Dawson, S. Rehal, G.S. Kibiki, G. Churchyard, I. Sanne, N.E. Ntinginya, L.T. Minja, R.D. Hunt, S. Charalambous, M. Hanekom, H.H. Semvua, S.G. Mpagama, C. Manyama, B. Mtafya, K. Reither, R.S. Wallis, A. Venter, K. Narunsky, A. Mekota, S. Henne, A. Colbers, G.P. van Balen, S.H. Gillespie, P.P.J. Phillips, and M. Hoelscher. 2017. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. *Lancet Infect. Dis.* 17: 39-49.
59. Ralph A.P., E. Kenangalem, G. Waramori, G.J. Pontororing, Sandjaja, E. Tjitra, G.P. Maguire, P.M. Kelly, and N.M. Anstey. 2013. High morbidity during treatment and residual pulmonary disability in pulmonary tuberculosis: Under-recognised phenomena. *PLoS ONE* 8: e80302.
60. Miller, M. R., J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C. P. van der Grinten, P. Gustafsson, R. Jensen, D. C. Johnson, N. Macintyre, R. McKay, D. Navajas, O. F. Pedersen, R. Pellegrino, G. Viegi, and J. Wanger. 2005. Standardisation of spirometry. *Eur. Respir. J.* 26:319-338.

61. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. 2002. ATS statement: guidelines for the six-minute walk test. *Am. J. Respir. Crit. Care Med.* 166:111-117.
62. Jensen, L. A., J. E. Onyskiw, and N. G. Prasad. 1998. Meta-analysis of arterial oxygen saturation monitoring by pulse oximetry in adults. *Heart Lung* 27:387-408.
63. Pasipanodya, J.G., T.L. Miller, M. Vecino, G. Munguia, S. Bae, G. Drewyer, and S.E. Weis. 2007. Using the St. George respiratory questionnaire to ascertain health quality in persons with treated pulmonary tuberculosis. *Chest* 132:1591-1598.
64. Kastien-Hilka, T., B. Rosenkranz, B. Bennett, E. Sinanovic, and M. Schwenkglenks. 2016. How to evaluate health-related quality of life and its association with medication adherence in pulmonary tuberculosis - Designing a prospective observational study in South Africa. *Front. Pharmacol.* 7:125.
65. Falk, A., J.B. O'Connor, and P.C. Pratt. 1969. Classification of pulmonary tuberculosis. Diagnostic Standards and Classification of Tuberculosis National Tuberculosis and Respiratory Disease Association, New York. 68-76.
66. Alling, D.W. and E.B. Bosworth. 1960. The after-history of pulmonary tuberculosis. VI. The first fifteen years following diagnosis. *Am. Rev. Respir. Dis.* 81:839-849.
67. Willcox, P.A. and A.D. Ferguson. 1989. Chronic obstructive airways disease following treated pulmonary tuberculosis. *Respir. Med.* 83:195-198.
68. Ralph, A.P., M. Ardian, A. Wiguna, G.P. Maguire, N.G. Becker, G. Drogumuller, M.J. Wilks, G. Waramori, E. Tjitra, Sandjaja, E. Kenagalem, G.J. Pontororing, N.M. Anstey, and P.M. Kelly. 2010. A simple, valid, numerical score for grading chest x-ray severity in adult smear-positive pulmonary tuberculosis. *Thorax* 65:863-869.
69. Warner, D.F. and V. Mizrahi. 2014. Shortening treatment for tuberculosis--to basics. *N. Engl. J. Med* 371:1642-1643.
70. Ramachandran, A., C. Snehalatha, A. Yamuna, S. Mary, and Z. Ping. 2007. Cost-effectiveness of the interventions in the primary prevention of diabetes among Asian Indians: within-trial results of the Indian Diabetes Prevention Programme (IDPP). *Diabetes Care* 30:2548-2552.
71. Sivalingam, V.N., J. Myers, S. Nicholas, A.H. Balen, and E.J. Crosbie. 2014. Metformin in reproductive health, pregnancy and gynaecological cancer: established and emerging indications. *Hum. Reprod. Update.* 20:853-868.
72. Salpeter, S.R., E. Greyber, G.A. Pasternak, and E.E. Salpeter. 2010. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* CD002967.
73. Graham, G.G., J. Punt, M. Arora, R.O. Day, M.P. Doogue, J.K. Duong, T.J. Furlong, J.R. Greenfield, L.C. Greenup, C.M. Kirkpatrick, J.E. Ray, P. Timmins, K.M. Williams. 2011. Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.* 50:81-98.
74. Chandel, N.S., D. Avizonis, C.R. Reczek, S.E. Weinberg, S. Menz, R. Neuhaus, S. Christian, A. Haegebarth, C. Algire, M. Pollak. 2016. *Cell Metab.* 23: 569-570.
75. Williams, L.K., B. Padhukasahasram, B.K. Ahmedani, E.L. Peterson, K.E. Wells, E. Gonzalez Burchard, D.E. Lanfear. 2014. Differing effects of metformin on glycemic control by race-ethnicity. *J. Clin. Endocrinol. Metab.* 99:3160-3168.
76. Song, I.H., J. Zong, J. Borland, F. Jerva, B. Wynne, M.J. Zamek-Gliszczynski, J.E. Humphreys, G.D. Bowers, and M. Choukour. 2016. The effect of dolutegravir on the pharmacokinetics of metformin in healthy participants. *J. Acquir. Immune. Defic. Syndr.* 72:400-407.
77. Gervasoni, C., D. Minisci, E. Clementi, G. Rizzardini, and D. Cattaneo. 2017. How relevant is the interaction between dolutegravir and metformin in real life? *J. Acquir. Immune Defic. Syndr.* 75: e24-26.

78. Masich, A., M.E. Badowski, M.D. Liedtke, and P.P. Fulco. 2017. Evaluation of the concurrent use of dolutegravir and metformin in human immunodeficiency virus (HIV)-infected patients. *Int. J. STD AIDS*. 28: 1229-1233.
79. Te Brake, L.H.M., V. Yunivita, R. Livia, N. Soetedjo, E. van Ewijk-Beneken Kolmer, J.B. Koenderink, D.M. Burger, P. Santoso, R. van Crevel, B. Alisjahbana, R.E. Aarnoutse, R. Ruslami. 2019. Rifampicin alters metformin plasma exposure but not blood glucose levels in diabetic tuberculosis patients. *Clin. Pharmacol. Ther.* 105:730-737.
80. Grun, B., M. K. Kiessling, J. Burhenne, K. D. Riedel, J. Weiss, G. Rauch, W. E. Haefeli, and D. Czock. 2013. Trimethoprim-metformin interaction and its genetic modulation by OCT2 and MATE1 transporters. *Br. J. Clin. Pharmacol.* 76:787-796.
81. Pfyffer, G.E., H.M. Welscher, P. Kissling, C. Cieslak, M.J. Casal, J. Gutierrez, and S. Rusch-Gerdes. 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. *J. Clin. Microbiol.* 35:364-368.
82. Dawson, T.G., R. van Zyl-Smit, H. Mishra, E. Streicher, S. Murray, A. Whitelaw, M. Hoelscher, S. Sharma, M. Pai, R. Warren, and K. Dheda. 2011. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *Am. J. Respir. Crit. Care Med.* 184: 132-140.
83. van der Watt, J.J., M.G. Benatar, T.B. Harrison, H. Carrara, and J.M. Heckmann. 2015. Isoniazid exposure and pyridoxine levels in human immunodeficiency virus associated distal sensory neuropathy. *Int. J. Tuberc. Lung Dis.* 19:1312-1319.
84. Wulffele, M.G., A. Kooy, L.D. Bets, J.C. Ogterop, B. van der Burg, A.J.M. Donker, C.D. A. Stehouwer. 2003. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J. Intern. Med* 254:455-463. and vitamin B12 100 mg daily
85. Lalau, J.D. 2010. Lactic acidosis induced by metformin: incidence, management and prevention. *Drug Saf.* 33:727-740.
86. Rayman, G., P.R. Vas, N. Baker, C.G. Taylor, Jr., C. Gooday, A.I. Alder, and M. Donohoe. 2011. The Ipswich Touch Test: a simple and novel method to identify inpatients with diabetes at risk of foot ulceration. *Diabetes Care* 34:1517-1518.
87. Lettieri, C.J., S.D. Nathan, R.F. Browning, S.D. Barnett, S. Ahmad, and A.F. Shorr. 2006. The distance-saturation product predicts mortality in idiopathic pulmonary fibrosis. *Respir. Med* 100:1734-1741.
88. Levey, A.S., L.A. Stevens, C.H. Schmid, Y.L. Zhang, A.F. Castro, III, H.I. Feldman, J.W. Kusek, P. Eggers, L. F. Van, T. Greene, and J. Coresh. 2009. A new equation to estimate glomerular filtration rate. *Ann. Intern. Med* 150:604-612.
89. Booyesen, H.L., A.J. Woodiwiss, A. Raymond, P. Sareli, H.C. Hsu, P.H. Dessen, and G.R. Norton. 2016. Chronic kidney disease epidemiology collaboration-derived glomerular filtration rate performs better at detecting preclinical end-organ changes than alternative equations in black Africans. *J. Hypertens.* 34:1178-1185.
90. DeFronzo, R., G.A. Fleming, K. Chen, and T.A. Bicsak. 2016. Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism* 65:20-29.
91. Lazarus, B., A. Wu, J.I. Shin, Y. Sang, G.C. Alexander, A. Secora, L.A. Inker, J. Coresh, A.R. Chang, M.E. Grams. 2018. Association of metformin use with risk of lactic acidosis across a range of kidney function: a community-based cohort study. *JAMA Intern. Med.* 78:903-910.
92. World Health Organization 2006. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia. WHO Press, Geneva.
93. Moyo, S., H. Bussmann, P. Mangwendeza, P. Dusara, T. Gaolathe, M. Mine, R. Musonda, W.E. van, V. Novitsky, J. Makhema, R.G. Marlink, M. Essex, and C.W. Wester. 2011. Validation of A Point-of-Care Lactate Device For Screening At-Risk Adults Receiving Combination Antiretroviral Therapy In Botswana. *J. Antivir. Antiretrovir.* 3:45-48.

94. Perez, E.H., H. Dawood, U. Chetty, T. M. Esterhuizen, and M. Bizaare. 2008. Validation of the Accutrend lactate meter for hyperlactatemia screening during antiretroviral therapy in a resource-poor setting. *Int. J. Infect. Dis.* 12:553-556.
95. Brackett, C.C. 2010. Clarifying metformin's role and risks in liver dysfunction. *J. Am. Pharm. Assoc.* 50:407-410.
96. Doyle, M.A., J. Singer, T. Lee, M. Muir, and C. Cooper. 2016. Improving treatment and liver fibrosis outcomes with metformin in HCV-HIV co-infected and HCV mono-infected patients with insulin resistance: study protocol for a randomized controlled trial. *Trials* 17:331.
97. Zhang, X., W.S. Harmsen, T.A. Mettler, W.R. Kim, R.O. Roberts, T.M. Therneau, L.R. Roberts, and R. Chaiteerakij. 2014. Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. *Hepatology* 60:2008-2016.
98. Wallis, R.S. and T. Peppard. 2015. Early Biomarkers and Regulatory Innovation in Multidrug-Resistant Tuberculosis. *Clin. Infect. Dis.* 61Suppl 3:S160-S163.
99. Wallis, R.S., A. Vinhas, J.L. Johnson, F.C. Ribeiro, M. Palaci, R.L. Peres, R.T. Sa, R. Dietze, A. Chiunda, K. Eisenach, and J.J. Ellner. 2003. Whole blood bactericidal activity during treatment of pulmonary tuberculosis. *J. Infect. Dis.* 187:270-278.
100. Cheon S.H., B. Kampmann, A.G. Hise, M. Phillips, H.Y. Song, K. Landen, Q. Li, R. Larkin, J.J. Ellner, R.F. Silver, D.F. Hoft, and R.S. Wallis. 2002. Bactericidal activity in whole blood as a potential surrogate marker of immunity after vaccination against tuberculosis. *Clin. Diagnost. Lab. Immunol.* 9:901-907.
101. Hoft, D.F., S. Worku, B. Kampmann, C.C. Whalen, J.J. Ellner, C.S. Hirsch, R.B. Brown, R. Larkin, Q. Li, H. Yun, and R.F. Silver. 2002. Investigation of the relationships between immune-mediated inhibition of mycobacterial growth and other potential surrogate markers of protective Mycobacterium tuberculosis immunity. *J. Infect. Dis.* 186:1448-1457.
102. Kampmann, B., G.N. Tena, S. Mzazi, B. Eley, D.B. Young, M. Levin. 2004. Novel human in vitro system for evaluating antimycobacterial vaccines. *Infect. Immun.* 72:6401-6407.
103. Saliu, O.Y., C. Sofer, D.S. Stein, S.K. Schwander, and R.S. Wallis. 2006. Tumor-necrosis-factor blockers: differential effects on mycobacterial immunity. *J. Infect. Dis.* 194:486-492.
104. Martineau, A.R., R.J. Wilkinson, K.A. Wilkinson, S.M. Newton, B. Kampmann, B.M. Hall, G.E. Packe, R.N. Davidson, S. M. Eldridge, Z.J. Maunsell, S.J. Rainbow, J.L. Berry, and C.J. Griffiths. 2007. A single dose of vitamin D enhances immunity to mycobacteria. *Am. J. Respir. Crit. Care Med.* 176:208-213.
105. Fletcher, H.A., R. Tanner, R.S. Wallis, J. Meyer, Z. R. Manjaly, S. Harris, I. Satti, R.F. Silver, D. Hoft, B. Kampmann, K.B. Walker, H.M. Dockrell, U. Fruth, L. Barker, M.J. Brennan, and H. McShane. 2013. Inhibition of mycobacterial growth in vitro following primary but not secondary vaccination with Mycobacterium bovis BCG. *Clin. Vaccine Immunol.* 20:1683-1689.
106. Wallis, R.S., C. Wang, D. Meyer, and N. Thomas. 2013. Month 2 culture status and treatment duration as predictors of tuberculosis relapse risk in a meta-regression model. *PLoS One* 8:e71116.
107. Wallis, R.S., C. Wang, T.M. Doherty, P. Onyebujoh, M. Vahedi, H. Laang, O. Olesen, S. Parida, and A. Zumla. 2010. Biomarkers for tuberculosis disease activity, cure, and relapse. *Lancet Infect. Dis.* 10:68-69.
108. Wallis, R. S., W. Jakubiec, M. Mitton-Fry, L. Ladutko, S. Campbell, D. Paige, A. Silvia, and P.F. Miller. 2012. Rapid evaluation in whole blood culture of regimens for XDR-TB containing PNU-100480 (sutezolid), TMC207, PA-824, SQ109, and pyrazinamide. *PLoS One* 7:e30479.
109. Wallis, R.S., W.M. Jakubiec, V. Kumar, A.M. Silvia, D. Paige, D. Dimitrova, X. Li, L. Ladutko, S. Campbell, G. Friedland, M. Mitton-Fry, and P.F. Miller. 2010. Pharmacokinetics

- and whole-blood bactericidal activity against *Mycobacterium tuberculosis* of single doses of PNU-100480 in healthy volunteers. *J. Infect. Dis.* 202:745-751.
110. Hong, Y.C., C.P. O'Boyle, I.C. Chen, C.T. Hsiao, and J.T. Kuan. 2008. Metformin-associated lactic acidosis in a pregnant patient. *Gynecol. Obstet. Invest.* 66:138-141.
 111. Carroll, D.G. and K.W. Kelley. 2014. Review of metformin and glyburide in the management of gestational diabetes. *Pharm. Pract. (Granada.)* 12:528.
 112. Scale, T. and J.N. Harvey. 2011. Diabetes, metformin and lactic acidosis. *Clin. Endocrinol. (Oxf.)* 74:191-196.
 113. Wester, C.W., S.K. Eden, B.E. Shepherd, H. Bussmann, V. Novitsky, D.C. Samuels, S.L. Hendrickson, C.A. Winkler, S.J. O'Brien, M. Essex, R.T. D'Aquila, V. DeGruttola, and R.G. Marlink. 2012. Risk factors for symptomatic hyperlactatemia and lactic acidosis among combination antiretroviral therapy-treated adults in Botswana: results from a clinical trial. *AIDS Res. Hum. Retroviruses* 28:759-765.
 114. Aperis, G., C. Paliouras, A. Zervos, A. Arvanitis, and P. Alivannis. 2011. Lactic acidosis after concomitant treatment with metformin and tenofovir in a patient with HIV infection. *J. Ren Care* 37:25-29.
 115. Ortiz-Brizuela, E., S. Perez-Patrigion, C. Recillas-Gispert, and F.J. Gomez-Perez. 2015. Lactic acidosis complicating metformin and non-nucleoside reverse transcriptase inhibitor combination therapy: a smoldering threat in the post-HAART era. *Rev. Invest. Clin.* 67:273-274.
 116. Hashim, H., N.S. Sahari, S.M. Sazlly Lim, and F.K. Hoo. 2015. Fatal tenofovir-associated lactic acidosis: a case report. *Iran Red. Crescent. Med. J.* 17:e19546.
 117. Naccarato, M., D. Yoong, and I.W. Fong. 2017. Dolutegravir and metformin: a case of hyperlactatemia. *AIDS* 31:2176-2177.
 118. Cho, S.K., J.S. Yoon, M.G. Lee, D.H. Lee, L.A. Lim, K. Park, M.S. Park, and J.-Y. Chung. 2011. Rifampin enhances the glucose-lowering effect of metformin and increases OCT1 Mrna levels in healthy participants. *Clin. Pharmacol. Ther.* 89:416-21.
 119. Takasu, N., T. Yamada, H. Miura, S. Sakamoto, M. Korenaga, K. Nakajima, M. Kanayama. 1982. Rifampicin-induced early phase hyperglycemia in humans. *Am. Rev. Respir. Dis.* 125:23-27.
 120. Weber, I.B., L. Baker, J. Mnyaluza, M.J. Matjila, K. Barnes, and L. Blumberg. 2010. The burden of imported malaria in Gauteng Province. *S. Afr. Med. J.* 100:300-303.
 121. Namale, P.E., L.H. Abdullahi, S. Fine, M. Kamkuemah, R.J. Wilkinson, and G. Meintjes. 2015. Paradoxical TB-IRIS in HIV-infected adults: a systematic review and meta-analysis. *Future. Microbiol.* 10:1077-1099.
 122. Lai, R.P., G. Meintjes, and R.J. Wilkinson. 2016. HIV-1 tuberculosis-associated immune reconstitution inflammatory syndrome. *Semin. Immunopathol.* 38:185-198.
 123. Ravimohan, S., N. Tamuhla, S. J. Kung, K. Nfanyana, A. P. Steenhoff, R. Gross, D. Weissman, and G.P. Bisson. 2016. Matrix Metalloproteinases in Tuberculosis-Immune Reconstitution Inflammatory Syndrome and Impaired Lung Function Among Advanced HIV/TB Co-infected Patients Initiating Antiretroviral Therapy. *EBioMed.* 3:100-107.
 124. Meintjes, G., S.D. Lawn, F. Scano, G. Maartens, M.A. French, W. Worodria, J.H. Elliott, D. Murdoch, R.J. Wilkinson, C. Seyler, L. John, M.S. van der Loeff, P. Reiss, L. Lynen, E.N. Janoff, C. Gilks, and R. Colebunders. 2008. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet. Infect. Dis.* 8:516-523.
 125. Goldin, B.R., W. Li, K. Mansfield, M. Woods, C. Wanke, L. Freeman, A. Shevitz, L. Gualtieri, S. Bussell, and S.L. Gorbach. 2005. The effect of micronutrient supplementation on disease progression and death in simian immunodeficiency virus-infected juvenile male rhesus macaques. *J. Infect. Dis.* 192:311-318.

126. Lemon, S.C., M.L. Wang, N.M. Wedick, B. Estabrook, S. Druker, K. L. Schneider, W. Li, and L. Pbert. 2014. Weight gain prevention in the school worksite setting: results of a multi-level cluster randomized trial. *Prev. Med.* 60:41-47.
127. Lemon, S.C., J. Zapka, W. Li, B. Estabrook, M. Rosal, R. Magner, V. Andersen, A. Borg, and J. Hale. 2010. Step ahead a worksite obesity prevention trial among hospital employees. *Am. J. Prev. Med.* 38:27-38.
128. Pasipanodya, J.G., H. McIlleron, A. Burger, P.A. Wash, P. Smith, and T. Gumbo. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J. Infect. Dis.* 208: 1464-1473.
129. Chigutsa, E., J.G. Pasipanodya, M.E. Visser, P.D. van Helden, P.J. Smith, F.A. Sirgel, T. Gumbo, and H. McIlleron. 2015. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrob. Agents Chemother.* 59: 38-45.
130. Schoenfeld, D.A. and J.R. Richter. 1982. Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. *Biometrics* 38:163-170.
131. Piubello, A., S.H. Harouna, M.B. Souleymane, I. Boukary, S. Morou, M. Daouda, Y. Hanki, and D.A. Van. 2014. High cure rate with standardised short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. *Int. J. Tuberc. Lung Dis.* 18:1188-1194.
132. Kuaban, C., J. Noeske, H. L. Rieder, N. Ait-Khaled, J. L. Abena Foe, and A. Trebucq. 2015. High effectiveness of a 12-month regimen for MDR-TB patients in Cameroon. *Int. J. Tuberc. Lung Dis.* 19:517-524.
133. Benator, D., M. Bhattacharya, L. Bozeman, W. Burman, A. Cantazaro, R. Chaisson, F. Gordin, C.R. Horsburgh, J. Horton, A. Khan, C. Lahart, B. Metchock, C. Pachucki, L. Stanton, A. Vernon, M.E. Villarino, Y.C. Wang, M. Weiner, and S. Weis. 2002. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. *Lancet.* 360:528-534.
134. Smith, J.D., E. Mills, and S.E. Carlisle. 2016. Treatment of pediatric type 2 diabetes. *Ann. Pharmacother.* 50:768-777.
135. Montenegro, S.H., R.H. Gilman, P. Sheen, R. Cama, L. Caviedes, T. Hopper, R. Chambers, and R.A. Oberhelman. 2003. Improved detection of Mycobacterium tuberculosis in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. *Clin. Infect. Dis.* 36:16-23.
136. Maji A., R. Misra, A. Kumar Mondal, D. Kumar, D. Bajaj, A. Singhal, G. Arora, A. Bhaduri, A. Sajid, S. Bhatia, S. Singh, H. Singh, V. Rao, D. Dash, E. Baby Shalini, J. Sarojini Michael, A. Chaudhary, R.S. Gokhale, Y. Singh. 2015. Expression profiling of lymph nodes in tuberculosis patients reveal inflammatory milieu at site of infection. *Sci. Rep.* 5:15214.
137. Cheng C.Y., N.M. Gutierrez, M.B. Marzuki, X. Lu, T.W. Foreman, B. Paleja, B. Lee, A. Balachander, J. Chen, L. Tsenova, N. Kurepina, K.W.W. Teng, K. West, S. Mehra, F. Zolezzi, M. Poidinger, B. Kreiswirth, D. Kaushal, H. Kornfeld, E.W. Newell, A. Singhal A. 2017. Host sirtuin 1 regulates mycobacterial immunopathogenesis and represents a therapeutic target against tuberculosis. *Sci. Immunol.* 2:eaaj1789
138. Martinez, M., C.Y. Cheng, N. Ketheesan, A. Cullen, Y. Tang, J. Lum, K. West, M. Poidinger, D.A. Guertin, A. Singhal, H. Kornfeld. 2019. mTORC2/Akt activation in adipocytes is required for adipose tissue inflammation in tuberculosis. *EBioMed.* 45:314-327.
139. Berry M.P., C.M. Graham, F.W. McNab, Z. Xu, S.A. Bloch, T. Oni, K.A. Wilkinson, R. Banchereau, J. Skinner, R.J. Wilkinson, C. Quinn, D. Blankenship, R. Dhawan, J.J. Cush, A. Mejias, O. Ramilo, O.M. Kon, V. Pascual, J. Banchereau, D. Chaussabel, A. O'Garra. 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466:973-977.

22 APPENDICES

List of appendices in order:

Appendix A.

Figure 1, Study flow for the treatment arms (page 109)

Figure 2, Study flow for the omics control group (page 110)

Appendix B.

Table 1, Schedule of Interventional Study Procedures/Evaluations (page 111)

Table 2, Schedule of Omics Control Group Procedures/Evaluations (page 111)

Table 3, Toxicity Management Algorithm (pages 113-114)

Appendix C. Pharmacy Manual (pages 115-126)

Appendix D. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, (pages 127-159)

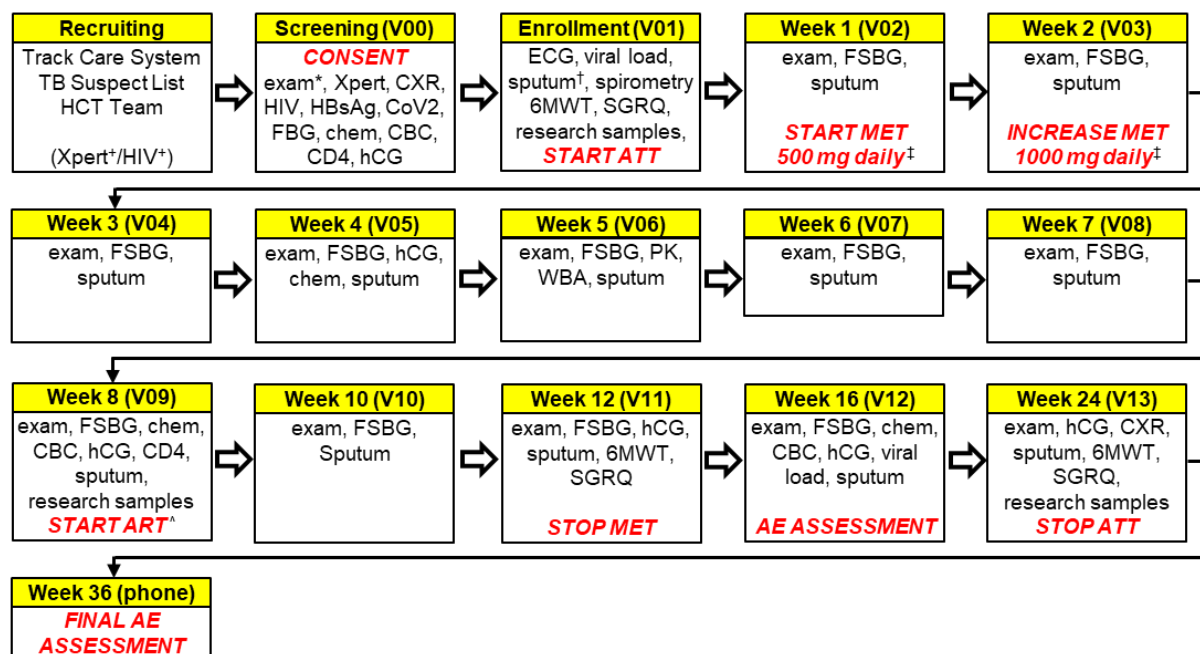
Appendix E. Adverse Event Processing, SOP-IEC-005v6 (pages 160-168)

Appendix F. SAHPRA document Reporting Adverse Drug Reactions in South Africa version 1_2 (pages 169-186)

Appendix G. Metformin package insert (pages 187-215)

Appendix H. Radiographic Scoring Manual (pages 216-220)

Appendix A, Figure 1. Study flow for the treatment arms.



Abbreviations: 6MWT, 6-minute walk test; ART, antiretroviral therapy; ATT, anti-TB treatment; chem, electrolytes, creatinine and hepatic function panel; CoV2, SARS-CoV-2 PCR test; CXR, chest x-ray; FBG, fasting blood glucose; FSBG, fingerstick glucose (metformin arm only); hCG, pregnancy test (females); HBsAg, hepatitis B surface antigen; HIV, HIV serology; MET, metformin; PK, pharmacokinetics samples; SGRQ, St. George respiratory questionnaire; WBA, whole blood bactericidal assay; Xpert, sputum Xpert MTB/RIF.

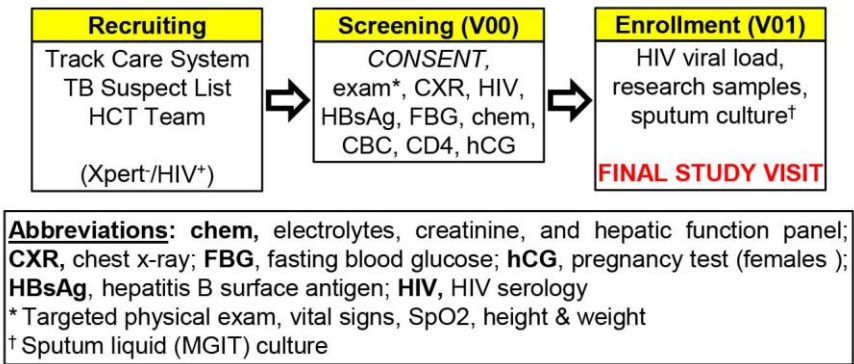
* Full physical exam at screening, targeted exam thereafter; vital signs, SpO2, weight on every visit (measure height on V00)

† Sputum liquid (MGIT) cultures at all indicated times; solid cultures at enrollment and week-8

‡ Metformin arm receives 500 mg tablet daily with meals for one week, then one 500 mg tablet twice daily for ten weeks

^ Latest ART initiation date for participants who were HIV treatment-naïve at enrollment

Appendix A, Figure 2. Study flow for the omics control group.



Appendix B, Table 1. Schedule of Interventional Study Procedures/Evaluations

	Screen	Enroll													
Visit	00	01	02	03	04	05	06	07	08	09	10	11	12	13	Phone
Week		0	1	2	3	4	5	6	7	8	10	12	16	24	36
Assess eligibility	X	X													
Consent	X														
Intervention ¹			500	1000	1000	1000	1000	1000	1000	1000	1000	STOP			
ATT		X	X	X	X	X	X	X	X	X	X	X	X	STOP	
ART ²										X	X	X	X	X	
Weight ³	X			X	X	X	X	X	X	X	X	X	X	X	
Physical exam ⁴	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
hCG ⁵	X					X				X		X	X	X	
Blood glucose ⁶	X			X	X	X	X	X	X	X	X	X	X	X	
Chemistries ⁷	X					X				X			X		
CBC	X									X			X		
CD4	X									X					
HIV viral load		X											X		
HIV POC serol ⁸	X														
HBsAg	X														
SARS-CoV-2 PCR	X														
ECG		X													
Sputum Xpert MTB/RIF	X														
Sputum culture ⁹		X	X	X	X	X	X	X	X	X	X	X	X	X	
Chest x-ray	X													X	
Spirometry		X										X		X	
6MWT ¹⁰		X										X		X	
SGRQ ¹¹		X										X		X	
Research samples ¹²		X								X				X	
PK samples ¹³							X								
WBA ¹⁴							X								
AE Assessment														X	X
Blood vol. (mL)	15.5	32.0	0	0	0	2.5	20.0	0	0	35.5	0	0	7.5	29.0	0

¹ Metformin 500 mg once daily x 1 wk, then 500 mg twice daily through week-12 (V12) for metformin arm participants only

² Participants on ART at enrollment will continue it; ART will start no later than week-8 for HIV treatment-naïve participants

³ Weight & height will be measured at screening to calculate BMI; weight will be repeated at subsequent visits

⁴ Full physical exam at screening and targeted exams on follow-up visits as described in Protocol

⁵ Females only

⁶ Fasting glucose by lab for all participants on V00; random glucose by glucometer on the indicated subsequent visits for metformin arm only

⁷ Includes electrolytes, creatinine and hepatic function panel (AST, ALT, ALP, total & direct bilirubin)

⁸ Uni-Gold and Determine point of care test strip kits

⁹ MGIT liquid sputum cultures at all indicated visits, with two MGIT cultures and solid culture at baseline and week-8 (V01 & V07)

¹⁰ Six-minute walk test, recording pre and post oximetry

¹¹ St. George's Respiratory Questionnaire

¹² Plasma, serum, PBMC, RNA (DNA will be isolated from blood samples and stored on site at the Tembisa CRC)

¹³ Intensive PK sampling (14.0 mL) in 20 participants; sparse PK sampling in all others (6.0 mL)

¹⁴ Whole blood bactericidal assay (6.0 mL) on 40 participants in the sparse PK sampling group

Appendix B, Table 2. Schedule of Omics Control Group Procedures/Evaluations.

	Screen	Enroll
Visit	00	01
Assess eligibility	X	X
Consent	X	
Height & weight	X	
Physical exam	X	X
hCG ¹	X	
Fasting blood glucose	X	
Chemistries ²	X	
CBC	X	
CD4	X	
HIV viral load		X
HIV POC serol ³	X	
HBsAg	X	
SARS-CoV-2 PCR	X	
Sputum culture ⁴		X
Chest x-ray	X	
Research samples ⁵		X
Blood vol. (mL)	15.5	32.0

¹ Females only

² Electrolytes, creatinine, hepatic function panel

³ Uni-Gold and Determine point of care test strips

⁴ MGIT liquid sputum culture

⁵ Plasma, serum, PBMC, RNA

Appendix B, Table 3. Toxicity Management Algorithm

Toxicities potentially caused by metformin vs ATT (INH, RIF, PZA, and EMB) or ART (TDF, FTC, and EFV). All grade 4 toxicities will be study endpoints whether judged related to metformin or not.

Signs/symptoms & evaluations	Other potential causal Agent	Management Strategy		
		Grade 1	Grade 2	Grade 3
Asthenia		Check for hypoglycemia and LA; if evaluations negative, then observe	Stop study drug 3 days and reevaluate	Same as Grade 2
Headache, dizziness, sleep disturbance	TDF, FTC, EFV, DTG	Observe	Same as Grade 1	Hold study drug 3 days and reevaluate
Nausea, vomiting, diarrhea	INH, RIF, PZA, TDF, FTC, EFV, DTG	Review dose timing; observe	Stop study drug 3 days and reevaluate	Same as Grade 2
Flu-like symptoms	RIF	Observe	Same as Grade 1	Stop study drug 3 days and reevaluate
Peripheral neuropathy, paresthesia's, weakness, tingling, numbness	INH	Review adherence with vitamin B6; observe	Same as Grade 2	Stop study drug for 7 days, neurology consultation
Weight loss	INH, RIF, PZA, TDF, FTC, EFV	Review dose timing and food insecurity; stop study drug if weight <10% of baseline and reevaluate in 14 days	Same as Grade 1	Same as Grade 1
Hypersensitivity (rash, itching, urticaria)	INH, PZA, RIF, EMB, TDF, FTC, EFV, DTG	If no urticaria, continue study drug, ATT, ART and treat with Benadryl PRN and observe; if urticarial present, same as Grades 2 and 3.	Stop study drug, ATT and ART; if rash improved in 7 days, restart ATT sequentially per ATS guidelines ⁹⁶ , then ART, then study drug	Same as Grade 2
Hepatic dysfunction	INH, PZA, RIF, FTC, TDF, EFV, DTG	If asymptomatic and AST or ALT <5X ULN, continue treatment and re-check HFP; check other causes including alcohol and acetaminophen; if AST or ALT ≥5X ULN, same as Grades 2 and 3	If ALP and bilirubin are disproportionately increased, stop RIF and re-check LFTs; For AST or ALT ≥3X ULN, stop study drug, ATT and ART drugs; if AST/ALT improves to ≤2X ULN then restart ATT sequentially per ATS guidelines ⁹⁶ , then ART, then study drug	Same as Grade 2
Hypoglycemia (blood glucose < 3.9 mmol/L)		Treat immediately with 20 gm glucose PO; confirm glucometer reading	Same as Grade 1	Same as Grade 1 but treat severely symptomatic

		with plasma glucose; review food insecurity and timing of study drug dose with meals; stop study drug and trend FPG for 3 days, then resume study drug and trend FPG for 3 days		hypoglycemia glucagon 1 mg SC
Symptomatic hyperlactatemia (lactate >4.4 mmol/L)		Stop study drug; check for acidosis and for other causes of lactate elevation (Section 13.1); challenge with study drug after 3 days if lactate <4.4 mmol/L	Same as Grade 1	Same as Grade 1
Lactic acidosis	TDF, FTC, EFV, DTG	Stop study drug; check for other causes of lactate acidosis; evaluate need for transfer to Zamokhule Hospital or Jose Pearson TB hospital.	Same as Grade 1	Same as Grade 1